Hemirubin: An Intramolecularly Hydrogen-Bonded Analogue for One-Half Bilirubin

Qingqi Chen and David A. Lightner*

Department of Chemistry, University of Nevada, Reno, Nevada 89557-0020

Received December 9, 1997

A model for one-half bilirubin, the neurotoxic yellow-orange pigment of jaundice, 9-[2-(2-carboxyethyl)benzyl]-2,3,7,8-tetramethyl-1,10-dihydrodipyrrin (**1**, hemirubin) was synthesized following $SnCl_4$-catalyzed Friedel−Crafts acylation at C(9) of 2,3,7,8-1-oxo-1,10-dihydrodipyrrin (**7**) with methyl *o*-(chlorocarbonyl)hydrocinnamate. Unlike earlier bilirubin model compounds, hemirubin is predicted and found to engage in intramolecular hydrogen bonding. Like bilirubin, the propionic acid carboxyl group of hemirubin is linked to the opposing dipyrrinone by intramolecular hydrogen bonds, and thus, **1** shares in some of the solution properties of its parent bilirubin, e.g., an acid that is less polar than its methyl ester.

Introduction

Typical dipyrrinones are bright yellow compounds with an intense UV−visible absorption near 400 nm ($\epsilon \sim 30{,}000$ L mol^{-1} cm^{-1}) due to a long axis-polarized $\pi \rightarrow \pi^*$ excitation in the 14π electron conjugated chromophore (Figure 1).[1] They are known from ^{15}N NMR[1], X-ray crystallography,[1,2] and molecular mechanics calculations[1,3] to prefer the lactam tautomer and the *Z*-configuration C=C at C(4) and to show substantial double-bond and single-bond character in the C(4)−C(5) and C(5)−C(6) bonds, respectively. The dipyrrinone chromophore is found in nature in tetrapyrrole bile pigments, most notably in (4*Z*,15*Z*)-bilirubin-IXα (Figure 1), the end product of heme metabolism in mammals and colorful herald of hepatobiliary disease.[4] Bilirubin, the yellow-orange pigment of jaundice,[5] has two dipyrrinones, each with a single propionic acid. These dipyrrinones are conjoined at and capable of independent rotations about a $-CH_2-$ group, and it is through such rotations that the propionic carboxyl group on each dipyrrinone is brought into sufficiently close proximity to engage the other dipyrrinone's lactam and pyrrole moieties in a matrix of intramolecular hydrogen bonds (Figure 1). Collectively, these six hydrogen bonds act as a potent stabilizer of bilirubin conformation forcing it to adopt a ridge-tile shape[6] and controlling its solution, spectroscopic, and metabolic properties.

Whether in bilirubin or as independent units, dipyrrinones are known to be avid participants in hydrogen bonding.[1] They adopt essentially planar conformations

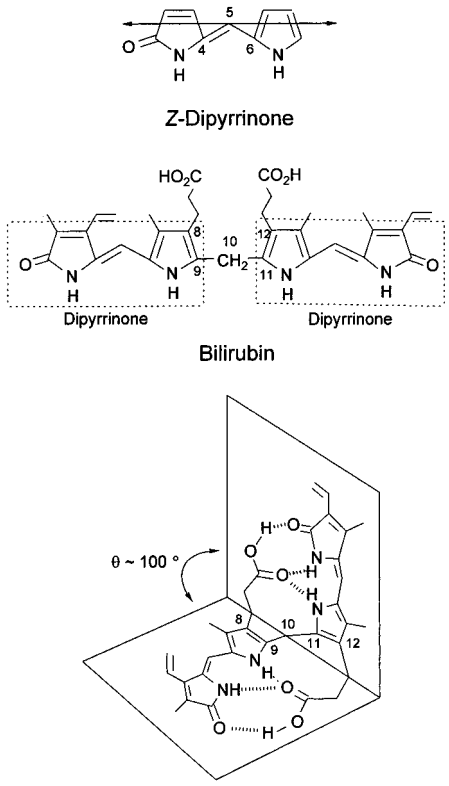
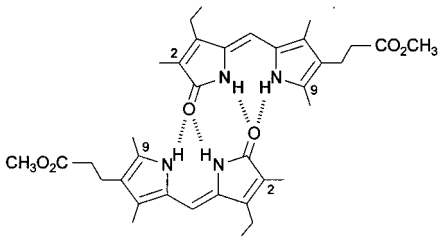


Figure 1. (Top) dipyrrinone chromophore. The double-headed arrow approximates the long axis polarization of the intense ~400 nm electronic transition. (Middle) bilirubin in the unstable linear representation is composed of two dipyrrinone chromophores. (Bottom) most stable bilirubin conformation shaped like a ridge-tile with hydrogen bonding between carboxylic acid groups and opposing dipyrrinones shown by dashed lines.

(torsion angle C(4)−C(5)−C(6)−N ~0°) in the crystal, where they are present as intermolecularly hydrogen-bonded planar dimers (Figure 2).[1,2] Such dimers persist in solutions of nonpolar solvents. In $CHCl_3$, for example, dipyrrinones are strongly associated with dimerization constants of 1700 M (37 °C) for kryptopyrromethenone[7] and 25 000 M (22 °C) for methyl xanthobilirubinate,[8] as measured by vapor-pressure osmometry and ^1H NMR

(1) For leading references, see: Falk, H. *The Chemistry of Linear Oligopyrroles and Bile Pigments*; Springer-Verlag: New York, 1989.
(2) (a) Cullen, D. L.; Black, P. S.; Meyer, E. F., Jr.; Lightner, D. A.; Quistad, G. B.; Pak, C.-S. *Tetrahedron* **1977**, *33*, 477−483. (b) Cullen, D. L.; Pèpe, G.; Meyer, E. F., Jr.; Falk, H.; Grubmayr, K. *J. Chem. Soc., Perkin Trans 2* **1979**, 999−1004. (c) Gossauer, A.; Blacha, M.; Sheldrick, W. S. *J. Chem. Soc., Chem. Commun.* **1976**, 764−765. (d) Sheldrick, W. S.; Borkenstein, A.; Blacha-Puller, M.; Gossauer, A. *Acta Crystallogr.* **1977**, *B33*, 3625−3635. (e) Sheldrick, W. S. *Israel J. Chem.* **1983**, *23*, 155−166.
(3) Falk, H.; Müller, N. *Tetrahedron* **1983**, *39*, 1875−1885.
(4) Ostrow, J. D., Ed. *Bile Pigments and Jaundice*; Marcel-Dekker: New York, 1986; and references therein.
(5) McDonagh, A. F.; Lightner, D. A. *Pediatrics* **1985**, *75*, 443−455.
(6) Person, R. V.; Peterson, B. R.; Lightner, D. A. *J. Am. Chem. Soc.* **1994**, *116*, 42−59.

S0022-3263(97)02227-5 CCC: $15.00
Published on Web 03/19/1998



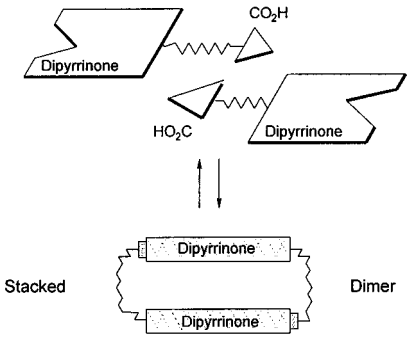


Figure 2. Dipyrrinone dimers. (Top) most stable dimer of methyl xanthobilirubinate with four intermolecular hydrogen bonds. Consistent with this dimeric representation, ^{1}H NMR NOEs measured in $CDCl_3$ are found between the methyls at C(2) and C(9) (ref 8). (Bottom) schematic representation for formation of the most stable dimer of xanthobilirubic acid. The dipyrrinones are stacked, thereby allowing the formation of six intermolecular hydrogen bonds, as in bilirubin (Figure 1) and alleviating a nonbonded steric repulsion between the methyls at C(9).

spectroscopy, respectively. The dimers are held together by a matrix of four intermolecular hydrogen bonds, with a calculated stabilization enthalpy of 20−30 kcal/mol.[9] While a coplanar dipyrrinone-to-dipyrrinone motif (Figure 2) is probably the most common type of hydrogen bonding in dipyrrinone dimers[1] (and is found even in bilirubin dimethyl ester[1,10a,11]), when carboxylic acid groups are present another type of dimer can become more stable. Thus, unlike simple dipyrrinones and dipyrrinone esters, dipyrrinone acids may form π-facial stacked dimers where each carboxylic acid group makes three hydrogen bonds with a dipyrrinone, as was found recently in xanthobilirubic acid and its homologues.[12] These dipyrrinone acids eschew the planar dimer and its

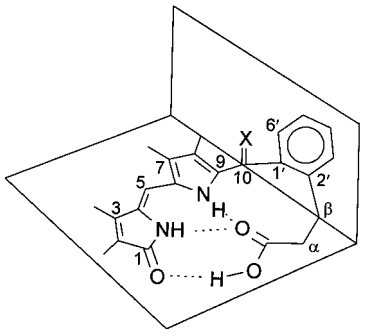


Figure 3. Folded, intramolecularly hydrogen-bonded conformation of hemirubin (**1**, X = H_2) and its 10-oxo analogue (**2**, X = O). Hydrogen bonds are represented by dashed lines. The shape is similar to one half of bilirubin (Figure 1).

four intermolecular hydrogen bonds in favor of gaining the added stabilization due to a total of six hydrogen bonds in the stacked dimer (Figure 2). This preference of dipyrrinones for hydrogen bonding to available carboxyl groups is not surprising since the planar dipyrrinones of bilirubin are tightly hydrogen bonded, albeit *intra*molecularly, to neighboring carboxylic acid groups (Figure 1).

In bilirubin and its analogues the component dipyrrinones participate in a unique type of hydrogen bonding with carboxylic acid groups (Figure 1), as confirmed in the solid by X-ray crystallography[2c,13] and in solution by ^{13}C{^{1}H} heteronuclear Overhauser effects[14] and ^{1}H NMR spectroscopy[10−12] and supported by molecular orbital and molecular dynamics computations.[6,15] Until recently,[16,17] bilirubin represented the only well-established example of carboxylic acid to amide hydrogen bonding. In the following, we show how a single dipyrrinone with only one propionic acid may be designed and constructed to engender *intra*molecular hydrogen bonding of the type characteristic of bilirubin. We call the new pigment hemirubin (**1**, Figure 3).

Results and Discussion

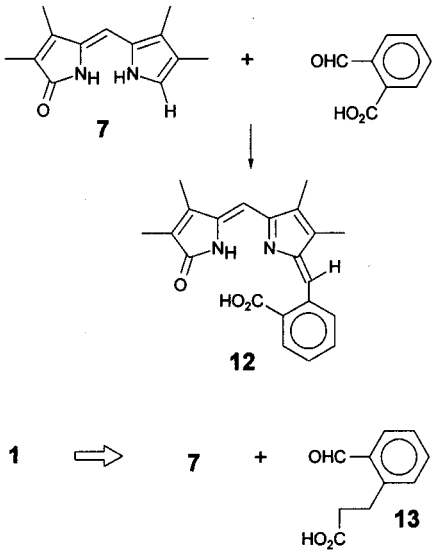
Synthesis. Falk et al.[18] had previously reported on the condensation of 2,3,7,8-tetramethyldipyrrinone (**7**) with *o*-formylbenzoic acid to afford the corresponding benzaldipyrrinone (**12**) (Scheme 1), and we had doubly

(7) Falk, H.; Grubmayr, K.; Höllbacher, G.; Hofer, O.; Leodolter, A.; Neufingerl, E.; Ribó, J. M. *Monatsh. Chem.* **1977**, *108*, 1113−1130.
(8) Nogales, D. F.; Ma, J.-S.; Lightner, D. A. *Tetrahedron* **1993**, *49*, 2361−2372.
(9) Molecular Mechanics calculations and molecular modeling were carried out on an Evans and Sutherland ESV-10 workstation using version 6.0 of SYBYL (Tripos Assoc., St. Louis, MO) as described in ref 6. The ball-and-stick drawings were created from the atomic coordinates of the molecular dynamics structures using Müller and Falk's "Ball and Stick" program for the Macintosh.
(10) (a) Kaplan, D.; Navon, G. *Israel J. Chem.* **1983**, *23*, 177−186. (b) Kaplan, D.; Navon, G. *Biochem. J.* **1982**, *201*, 605−613. (c) Navon, G.; Frank, S.; Kaplan, D. *J. Chem. Soc., Perkin Trans 2* **1984**, 1145−1149.
(11) Trull, F. R.; Ma, J. S.; Landen, G. L.; Lightner, D. A. *Israel J. Chem.* **1983**, *23*, 211−218.
(12) (a) Boiadjiev, S. E.; Anstine, D. T.; Lightner, D. A. *J. Am. Chem. Soc.* **1995**, *117*, 8727−8736. (b) Boiadjiev, S. E.; Anstine, D. T.; Maverick, E.; Lightner, D. A. *Tetrahedron: Asymmetry* **1995**, *6*, 2253−2270.
(13) (a) Bonnett, R.; Davies, J. E.; Hursthouse, M. B.; Sheldrick, G. M. *Proc. R. Soc. London, Ser. B* **1978**, *202*, 249−268. (b) LeBas, G.; Allegret, A.; Mauguen, Y.; DeRango, C.; Bailly, M. *Acta Crystallogr., Sect. B* **1980**, *B36*, 3007−3011. (c) Becker, W.; Sheldrick, W. S. *Acta Crystallogr., Sect. B* **1978**, *B34*, 1298−1304. (d) Mugnoli, A.; Manitto, P.; Monti, D. *Acta Crystallogr., Sect. C* **1983**, *38*, 1287−1291.
(14) (a) Nogales, D.; Lightner, D. A. *J. Biol. Chem.* **1995**, *270*, 73−77. (b) Dörner, T.; Knipp, B.; Lightner, D. A. *Tetrahedron* **1997**, *53*, 2697−2716.
(15) (a) Falk, H. Molecular Structure of Bile Pigments. In *Bilirubin*; Heirwegh, K. P. M., Brown, S. B., Eds.; CRC Press: Boca Raton, FL, 1982; Vols. 1 and 2, pp 7−29 and references therein. (b) Shelver, W. H.; Rosenberg, H.; Shelver, W. H. *Intl. J. Quantum Chem.* **1992**, *44*, 141−163. (c) Shelver, W. L.; Rosenberg, H.; Shelver, W. H. *J. Mol. Struct.* **1994**, *312*, 1−9.
(16) Wash, P. L.; Maverick, E.; Chiefari, J.; Lightner, D. A. *J. Am. Chem. Soc.* **1997**, *119*, 3802−3806.
(17) (a) Garcia-Tellado, F.; Gaswami, S.; Chang, S.-K.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1990**, *112*, 7393−7394. (b) Hamann, B. C.; Branda, N. R.; Rebek, J., Jr. *Tetrahedron Lett.* **1993**, *34*, 6837−6840. (c) Kelly, T. R.; Kim, M. H. *J. Am. Chem. Soc.* **1994**, *116*, 7072−7080.
(18) Eichinger, D.; Falk, H. *Monatsh. Chem.* **1987**, *118*, 91−103, 261−271.

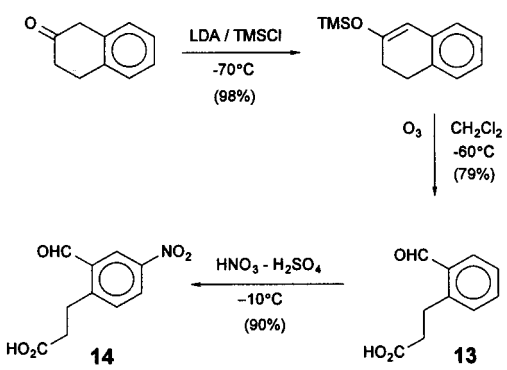
Table 1. Attempted Condensations of Dipyrrinone 7 with Benzaldehyde 13 To Give the Benzaldipyrrinone

catalyst	solvent	*T* (°C)	rctn time	results (% yield)
HBr−HOAc	CH_2Cl_2	25	2 min	**15** (100)
HBr−HOAc	CH_2Cl_2	0	1 min	**15** (100)
HBr−HOAc	CH_2Cl_2	−20	3 h	**15** (60) and unreacted **13**
HBr−HOAc	CH_2Cl_2	−60	12 h	**15** (80) and unreacted **13**
TFA	none	rt	3 h	**15** (100)
TFA	CH_2Cl_2	−20	3 h	**15** (50) and recovered **13**
TFA	MeOH	−20	1 h	**15** (30) and recovered **13**
TsOH	CH_2Cl_2	rt	5 h	**15** (80)
TsOH	MeOH	rt	12 h	**15** (60)
TsOH	CH_2Cl_2-MeOH	rt	10 h	**15** (60)
TsOH	none	rt	10 h	**15** (75)
AcOH	CH_2Cl_2	rt	2 h	**15** (63) and recovered **13**
AcOH	CH_2Cl_2-MeOH	rt	30 h	**15** (80)
none	CH_2Cl_2	rt	5 h	**15** (30) and recovered **13**
none	MeOH	reflux	2−10 h	NR recovered **7**
Bu_4NOH	MeOH-THF	reflux	2 h	NR

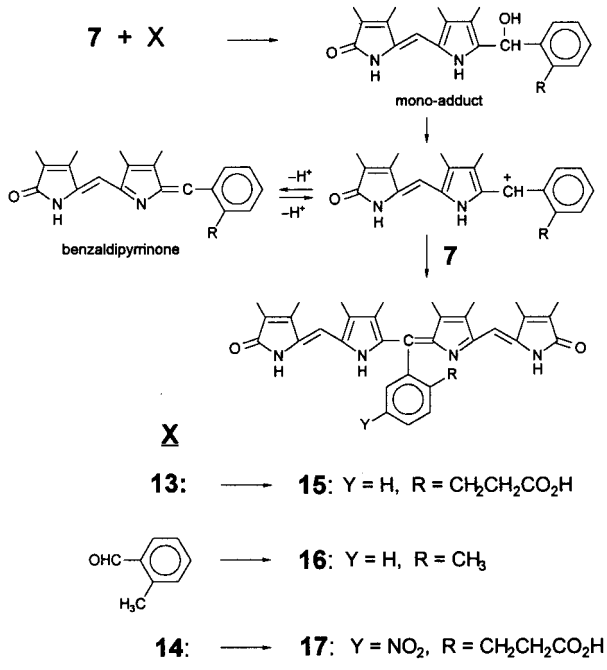
Scheme 1



Scheme 2



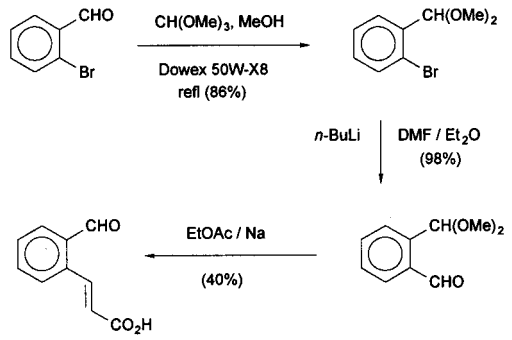
Scheme 3



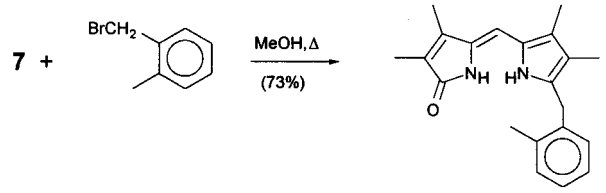
condensed the same dipyrrinone with the aromatic dialdehydes, terephalaldehyde or isophthalaldehyde, to afford the corresponding tetrapyrrole dibenzalbisdipyrrinones.[19] Thus, with the expectation benzlpyrrolinones can be prepared readily from aromatic aldehydes and α-free dipyrrinones, we envisioned the preparation of **1** to follow from a straightforward coupling of dipyrrinone **7** with *o*-formylhydrocinnamic acid or ester (**13**) (Scheme 1). *β*-Alkylated dipyrrinones are relatively easy to synthesize, especially the 2,3,7,8-tetramethyldipyrrinone **7**,[20,21] and *o*-formylhydrocinnamic acid (**13**) can be prepared in high yield from *β*-tetralone (Scheme 2). Unexpectedly, however, reaction of **7** with **13** under reaction conditions that gave **12** did not stop at the monoadduct stage and form a stable benzaldipyrrinone (Scheme 3). Apparently, the initially formed monoadduct did not undergo smooth elimination to yield a stable benzaldipyrrinone. Either through protonation of the erstwhile benzalpyrrolinone or by elimination of water from a protonated aldol-like intermediate, the resulting carbocation attacked a second molecule of **7** to afford mainly verdin **15** and only a very small amount of the corresponding rubin, which was unstable and rapidly converted to **15** (Scheme 3). Extensive modification of the acid catalysis reaction conditions (Table 1) consistently afforded **15**, and we could isolate none of the desired monocondensation product. It would appear that attachment of the electron-withdrawing COOH group directly to the phenyl ring of the aldehyde (as in *o*-formylbenzoic acid) is responsible for the acid-catalyzed reaction of Scheme 1 having stopped at the single condensation stage, because reaction of **7**

(19) Nogales, D.; Anstine, D. T.; Lightner, D. A. *Tetrahedron* **1994**, *50*, 8579−8596.
(20) Montforts, F.-P.; Schwartz, U. M. *Liebigs Ann. Chem.* **1985**, 1228−1253.
(21) Xie, M.; Lightner, D. A. *Tetrahedron* **1993**, *49*, 2185−2200.

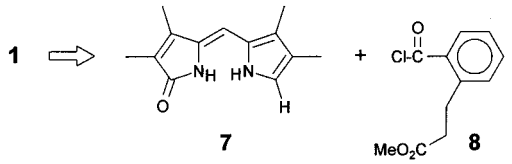
Scheme 4



Scheme 5



Scheme 6

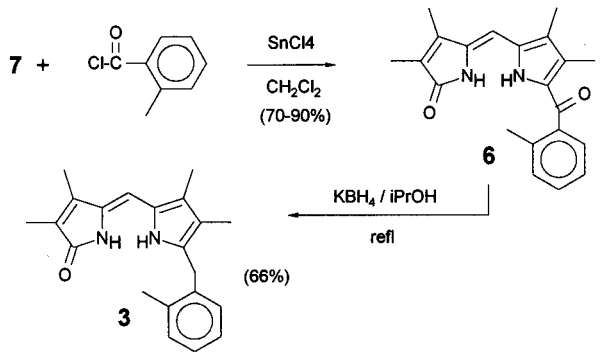


with *o*-tolualdehyde also gave only the verdin (**16**) (Scheme 3). Base catalysis gave no reaction. Surmising that the desired reaction required a deactivated benzaldehyde, we synthesized 2-methyl-5-nitrobenzaldehyde and found that it underwent smooth conversion in TFA to monocondensation product in 30% yield without formation of verdin. Unfortunately, for reasons that are not entirely clear, the more relevant nitrobenzaldehyde **14** (synthesized according to Scheme 2) gave only the verdin (**17**) in reaction with **7**. Similarly, reaction of *o*-formylcinnamic acid (prepared from *o*-bromobenzaldehyde according to Scheme 4) with **7** gave none of the target monocondensation product. In the midst of these difficulties, a different condensation reaction appeared promising: reaction of **7** with α-bromo-*o*-xylene was found to give a monocondensation product (Scheme 5). However, the reaction failed when *o*-(bromomethyl)hydrocinnamic acid methyl ester was reacted with **7** under the same conditions.

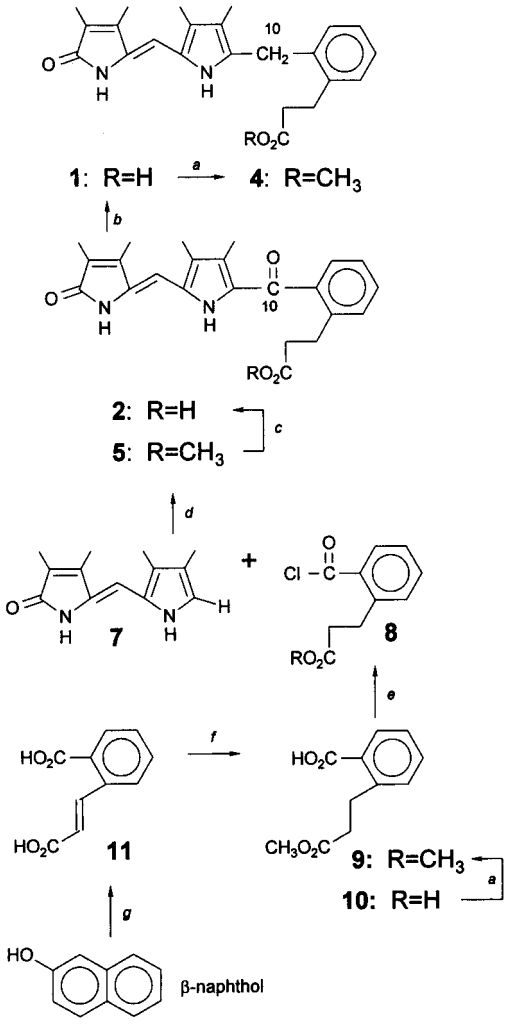
In view of the failure of the appropriate aldehyde or benzyl bromide condensations to give the target monocondensation products, an alternative route was sought wherein further reaction of the initial product would be unlikely: Friedel−Crafts acylation of dipyrrinone **7** with acid chloride **8** (Scheme 6). Friedel−Crafts-type acylations at an α- or *β*-free position of pyrroles and at *β*-free (8H) dipyrrinones have been reported,[22] but such reactions at an α-free (9H) dipyrrinone are less well-known. We found that the readily available *o*-methylbenzoyl chloride reacted with dipyrrinone **7** in good yield to afford the adduct (**6**, Scheme 7). Reduction of **6** to **3** failed with KBH_4 in methanol at reflux but proceeded smoothly in refluxing isopropyl alcohol. These positive results

(22) Thyrann, T.; Lightner, D. A. *Tetrahedron* **1996**, *52*, 447−460.

Scheme 7



Scheme 8. Synthetic Scheme[a]

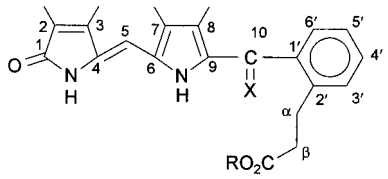


[a] Key: [a]CH_3OH/H_2SO, 77−85%; [b]$KBH_4/(CH_3)_2CHOH$ refl., 63−70%; [c]NaOH/CH_3OH, 75%; [d]$SnCl_4/CH_2Cl_2$, 70−95%; [e]$SOCl_2$, 66%; [f]Ni(Al)/NaOH, 86%; [g]30% CH_3CO_3H, 68%.

prompted us to proceed with the synthesis of **1** as outlined in the Scheme 8.

The required acid chloride **8** was prepared from *β*-naphthol. Oxidation with 30% peroxyacetic acid afforded *o*-carboxylcinnamic acid (**11**), which was reduced with Raney nickel alloy in aqueous NaOH to give **10**. The latter was selectively esterified in methanol−H_2SO_4 to afford **9**, from which acid chloride **8** was obtained following reaction with thionyl chloride. Ester−acid chloride **8** underwent a Friedel−Crafts acylation with dipyrrinone **7** (prepared as reported previously)[21] in

Table 2. ^{13}C NMR Chemical Shifts[a] of Dipyrrinone 7, Hemirubin 1 (X = H_2, R = H), Its Methyl Ester 4 (X = H_2, R = CH_3), *o*-Xylyldipyrrinone 3, and Related 9-Substituted Oxodipyrrinones 2 (X = O, R = H), 5 (X = O, R = CH_3), and 6



	dipyrrinone chemical shift in $(CD_3)_2SO$ and $(CDCl_3)$						
carbon	**7**	**1**	**4**	**3**	**2**	**5**	**6**[c]
1	172.3	171.8	172.1	173.2	173.0	172.6	173.0
(1)[b]	(174.3)	(174.4)	(173.6)	(173.2)	(173.3)	(173.2)	[172.8]
2	118.5	116.1	122.6	125.9	123.7	123.7	123.6
3	141.5	141.4	141.7	141.8	142.1	142.1	142.2
4	131.6	128.9	128.9	134.3	136.5	136.5	135.0
5	98.14	97.74	98.04	95.65	95.71	95.68	95.78
(5)[b]	(101.1)	(101.0)	(100.5)	(96.07)	(98.74)	(96.21)	[94.84]
6	124.5	123.5	125.5	131.2	130.9	127.4	127.8
7	124.1	122.7	125.4	126.2	126.5	126.6	126.1
8	122.0	122.3	123.0	126.1	127.3	127.3	127.0
9	120.1	128.4	128.9	135.0	130.3	128.0	134.7
10		28.45	29.00	29.43	186.0	185.9	186.2
(10)[b]		(25.58)	(27.65)	(30.52)	(177.6)	(186.3)	[185.9]
1′		137.5	137.8	135.4	141.0	141.0	141.26
2′		138.4	138.5	135.8	138.2	137.9	136.4
3′		126.0	126.6	130.0	128.0	129.9	129.7
4′		126.0	126.5	128.0	131.2	130.8	130.8
5′		126.0	126.1	127.9	129.8	129.9	127.3
6′		130.6	129.3	130.0	130.8	131.0	130.8
α		27.23	27.54	19.50	27.98	27.93	18.95
(α)[b]		(26.01)	(29.63)	(19.48)	(25.89)	(28.01)	[18.43]
β		34.04	34.12		35.31	35.03	
(*β*)[b]		(34.81)	(34.43)		(34.21)	(35.19)	
CO_2R		173.7	172.1		173.7	173.0	
(CO_2R)[b]		(178.9)	(173.6)		(175.5)	(173.5)	
2-CH_3	8.57	8.20	8.53	8.13	8.59	8.55	8.57
3-CH_3	9.36	9.46	9.78	10.33	9.78	9.77	9.78
7-CH_3	9.83	9.33	9.64	10.26	9.36	9.34	9.35
8-CH_3	10.26	8.73	9.06	9.42	10.78	10.77	10.49

[a] *δ*, ppm downfield from $(CH_3)_4Si$ for 10^{-2} M solutions in $(CD_3)_2SO$. [b] Values in parentheses are from 10^{-3} M solutions in $CDCl_3$. [c] Values in square brackets are from 10^{-3} M solutions in 10% $(CD_3)_2SO$−90% $CCDl_3$ by volume.

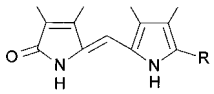
dichloromethane using $SnCl_4$ catalyst to give the benzoyldipyrrinone **5** in high yield. Saponification of **5** afforded the corresponding acid (**2**). Reduction of **5** to hemirubin **1** was achieved using KBH_4 in refluxing isopropyl alcohol. Thus, both hemirubin **1** and its 10-oxo analogue (**2**) could be obtained in a straightforward way. For comparison of spectroscopic properties, their methyl esters (**4**) and (**5**) were also prepared.

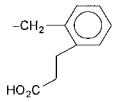
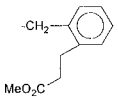
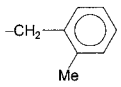
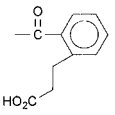
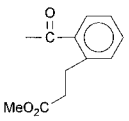
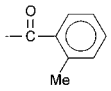
Molecular Structure. The constitutional structures of **1**−**6** (Schemes 7 and 8), which follow from the structure of the common starting dipyrrinone (**7**)[21] and the synthetic methods, were confirmed by their ^{13}C NMR spectra run in $(CD_3)_2SO$. In $(CD_3)_2SO$ solvent, **1**−**6** are expected to be monomeric and show chemical shifts (Table 2) characteristic of the dipyrrinone fragment and the *o*-substituted phenyl fragment—consistent with the postulated structures. In $CDCl_3$ solvent, where intramolecular hydrogen bonding is more likely to occur in **1** and **2**, and intermolecularly hydrogen-bonded dipyrrinone-to-dipyrrinone dimers are very probable in **3**−**7**, shifts were noted at particularly sensitive carbons (10, α, and CO_2R). In **1**, which is capable of exhibiting intramolecular hydrogen bonding, C(10) is strongly shielded in the spectrum run in $CDCl_3$, relative to that in $(CD_3)_2SO$, whereas in its ester (**4**) the shielding is only half as large. In the oxo-analogue (**2**) of **1**, the C(10) resonance found in $CDCl_3$ is also strongly shielded relative to its ester (**5**) and its *o*-toluoyldipyrrinone analogue (**6**). Similarly, the α-carbon resonance of **1** measured in $CDCl_3$ is more shielded than in $(CD_3)_2SO$, whereas in the methyl ester (**4**) it is more deshielded. The same observation is made at the α-carbon resonances of the oxo analogues **2** and **5**.

The geometric structural assignments, particularly the *Z*-configuration of the C(4) exocyclic double bond of the dipyrrinone moiety in **1**−**7**, is confirmed by the observation of strong homonuclear nuclear Overhauser effects (NOEs) in $CDCl_3$ between the lactam and pyrrole NHs and moderate NOEs between the C(5)−H and the C(3) and C(7) methyls. Other NOEs of significance were detected between the C(10) hydrogens of **1**, **3**, and **4** and (i) their benzene ring C(6) hydrogens, (ii) their pyrrole NHs, and (iii) their C(8) methyls. NOEs were found between the *β*-CH_2 of **1**−**4** and the benzene ring C(3′) hydrogens. *Significantly, as in bilirubin and mesobilirubin,[10,14b] weak NOEs were found between the carboxylic acid hydrogen and the lactam hydrogen in **1** and **2***. These data indicate a proximal spatial relationship between those groups that is consistent with the intramolecular hydrogen bonding motif shown in the structural representation of Figure 3.

Consistent with these observations, T_1 relaxation measurements of the CO_2H carbons in **1** and **2** were 3.31 and 3.21 s, respectively, in $CDCl_3$. In bilirubin, which is known to engage in intramolecular hydrogen bonding,

Table 3. Comparison of NH ^{1}H NMR Chemical Shifts[a] of Dipyrrinones in $CDCl_3$ and $(CD_3)_2SO$ Solvents



DIPYRRINONE R =		δ (ppm) in $CDCl_3$[b]			δ (ppm) in $(CD_3)_2SO$[b]		
		Lactam	Pyrrole	CO_2H	Lactam	Pyrrole	CO_2H
H	7	10.90	10.23	—	9.7	10.4	—
	1	10.55	8.99	13.42	9.72	10.27	12.17
	4	10.79	10.15	—	9.71	10.26	—
	3	10.90	10.01	—	9.73	10.29	—
	2	10.71	8.15	12.36	10.51	11.17	12.13
	5	9.57	9.36	—	10.46	11.13	—
	6	9.40	8.65	—	10.50	11.13	—

[a] δ, downfield from Me_4Si. [b] Run as 10^{-2} M $(CD_3)_2SO$ and 10^{-3} M $CDCl_3$ solutions at 23 °C.

T_1 for its CO_2H carbons is slightly longer (3.9 s), but methyl xanthobilirubinate, whose ester group does not engage in hydrogen bonding, has a much longer T_1. The data again are consistent with intramolecularly hydrogen-bonded carboxylic acid groups in **1** and **2**.

^{1}H NMR and Hydrogen Bonding. Dipyrrinones are avid hydrogen bonders.[1,8] Diagnostic of this behavior and typical of hydrogen bonding, the intrinsic N−H ^{1}H NMR chemical shifts of the monomer (δ ~8 ppm)[8] become strongly deshielded in nonpolar solvents such as $CDCl_3$ to approximately 10 and 11 ppm[11] for the pyrrole and lactam hydrogens, respectively. These more deshielded chemical shifts, found in a wide variety of dipyrrinones with hydrocarbon, ester, and amide substituents, are characteristic of the traditional dipyrrinone to dipyrrinone planar dimer (Figure 2, top)[1,8,11] However, when the dipyrrinones engage in hydrogen bonding to CO_2H groups to give a stacked dimer (Figure 2, bottom), the NH chemical shifts are relatively more shielded (especially pyrrole NH ~8.9 ppm, but also the lactam NH ~10.5 ppm). In tetrapyrroles, such as bilirubin, where the dipyrrinones are *intra*molecularly hydrogen bonded to the opposing propionic acids but are not stacked (Figure 1), the lactam and pyrrole chemical shifts lie near δ ~10.6 and 9.2, respectively, in $CDCl_3$. The N-*H* chemical shifts of hemirubin **1**, notably the shielded pyrrole NH near 9 ppm (Table 3), are similar to those of bilirubin, whereas those of hemirubin ester (**3**) and the *o*-xylyldipyrrinone **4** are more like those found in dipyrrinone **7**. The data point to intramolecular hydrogen bonding between dipyrrinone and lactam in hemirubin and intermolecular dipyrrinone-to-dipyrrinone hydrogen bonding in **3** and **4**. In $(CD_3)_2SO$ solvent, the N-*H* chemical shifts of **1**, **3**, **4**, and **7** are all very similar, indicative of hydrogen bonding to the solvent.

Similarly, for 10-oxohemirubin **2**, the N-*H* chemical shifts in $CDCl_3$ are indicative of intramolecular hydrogen bonding. In the ester (**5**), the pyrrole and lactam NH chemical shifts lie at 9.36 and 9.57 ppm, respectively, whereas in **2**, the corresponding chemical shifts lie at 8.15

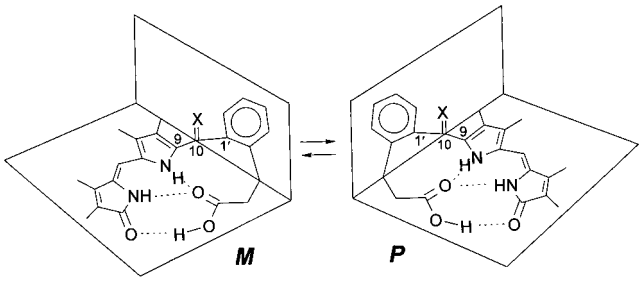


Figure 4. Interconverting, enantiomeric ridge-tile conformations of hemirubin (**1**, X = H_2) and 10-oxohemirubin (**2**, X = O). The ***M*** and ***P*** helicities are correlated with the negative and positive torsion angles [C(1′)−C(10)−C(9)−N], respectively. Hydrogen bonds are shown by dashed lines.

and 10.71 ppm—values consistent with intramolecular hydrogen bonding. Although the C(10) carbonyl causes some differences in NH chemical shifts relative to those of **1**, the strong shielding of the pyrrole NH is typical of a dipyrrinone hydrogen bonded to a carboxylic acid in an orientation where the pyrrole N-*H* is above or below the aromatic ring π-system. Such an arrangement is very much like that in ridge-tile bilirubin (Figure 1), and so it seems probable that hemirubin and its 10-oxo analogue fold into the conformation shown in Figure 3.

Like the bilirubin ridge-tile conformation, **1** and **2** may adopt either of two interconverting enantiomeric ridge-tile conformations (Figure 4). For bilirubin, the interconversion barrier has been determined experimentally to be ∼20 kcal/mol. The lowest energy interconversion pathway has been computed to be ∼20 kcal/mol over a transition state involving the breaking of at least three of the six intramolecular hydrogen bonds (while three hydrogen bonds are retained).[6] Interconversion of enantiomeric **1** or **2** also requires breaking three hydrogen bonds, but unlike the interconversion of bilirubin conformational enantiomers there are no residual 3 hydrogen bonds for use in stabilizing the transition state. The interconversion barrier is thus probably lower in the hemirubins, as supported by the observation that the ^{1}H NMR signals of the propionic $-C_\alpha H_2-C_\beta H_2-$ segment of **1** and **2** indicate a simple A_2B_2 splitting pattern. In contrast, bilirubin exhibits an ABMX splitting pattern characteristic of restricted segmental motion in the propionic acid chains on the NMR time scale.

Molecular Dynamics Calculations. In support of the conclusions reached (above) by NMR spectroscopic analysis, molecular dynamics calculations[9] of hemirubin (**1**) and its 10-oxo analogue (**2**) show that these compounds prefer intramolecularly hydrogen-bonded conformations (Figure 5), which are computed to lie some 13 kcal/mol lower in energy than the non-hydrogen-bonded forms. The intramolecularly hydrogen-bonded conformations shown in Figure 5 have torsion angles similar to those found in bilirubin and mesobilirubin.[6,13] The dipyrrinone moiety in **1** and **2** is twisted somewhat, with C(4)−C(5)−C(6)−N torsion angles of ∼18° and ∼29°, respectively. The C(1′)−C(10)−C(9)−N torsion angles are 64° and 24° in **1** and **2**, respectively—as compared to ∼60° in mesobilirubin.[6] In addition, the interplanar dihedral angles are 97° and 103° in **1** and **2**, respectively, as compared to ∼96° in mesobilirubin.[6] In sum, the molecular dynamics calculations suggest that **1** adopts a molecular geometry very similar to that found in bilirubin and mesobilirubin, whereas **2** is somewhat

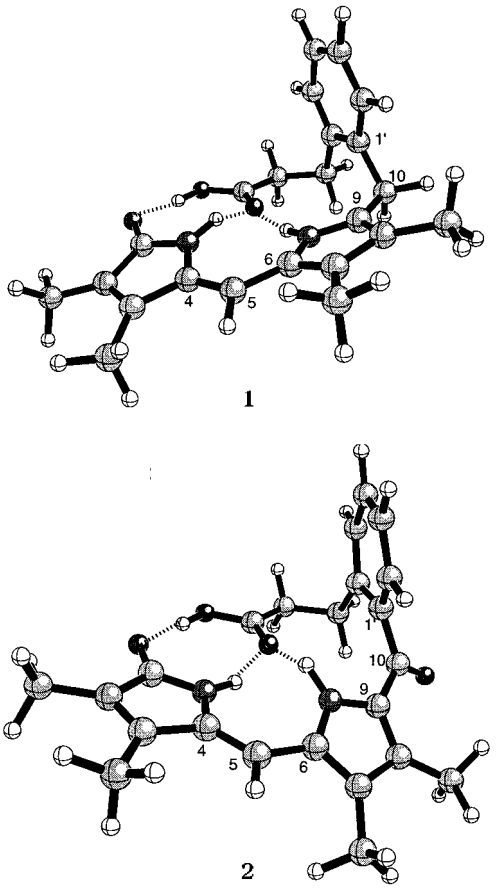


Figure 5. Ball and stick representations for the minimum energy conformations of hemirubin (**1**) and its 10-oxo analogue (**2**). The energy minimization and structure graphical representations were carried out according to refs 6 and 9. In **1**, the interplanar angle between the phenyl ring and average plane of the dipyrrinone is 97°, in **2** it is 103°. The C(4)−C(5)−C(6)−N torsion angles of **1** and **2** are 18° and 29°, respectively; the N−C(9)−C(10)−C(1′) torsion angles of **1** and **2** are 64° and 24°, respectively. The COOH···O=C (lactam) hydrogen bond distances are 1.553 and 1.535 Å in **1** and **2**, respectively; the HO−C=O···HN (lactam) hydrogen bond distances are 1.528 and 1.549 Å in **1** and **2**, respectively, and the HO−C=O···HN (pyrrole) hydrogen bond distances are 1.584 and 1.885 Å in **1** and **2**, respectively.

distorted from that geometry, presumably because its C(10) carbon is sp^2 hybridized rather than sp^3.

Optical Spectra. The UV−vis spectral data for **1**−**7** in solvents with a wide range of polarity are shown in Table 4. The long-wavelength absorption of hemirubin (**1**) has nearly the same λ^{max} and ϵ^{max} as its methyl ester (**4**) and is rather similar to its *o*-xylyl (**3**) analogue in methanol and in dimethyl sulfoxide, solvents likely to interfere with hydrogen bonding. In nonpolar solvents, however, the spectra of **1** and **4** (or **3**) are noticeably different, with **1** showing a strong bathochromic shift of λ^{max}, whereas λ^{max} of **3** and **4** are relatively unchanged. While the spectral shifts do not unambiguously confirm an intramolecularly hydrogen bonded structure for **1**, they lend support to the conclusions based on NMR spectral analysis and are consistent with the ability of **1** to adopt a unique conformational structure in nonpolar solvents. The UV−vis spectral data for **2** are less solvent dependent. The major long wavelength absorption near 400 nm has an inflection or shoulder near 420−430 nm in the various solvents of Table 4.

Table 4. Solvent Dependence of UV−vis Data for Hemirubin (1) and Related Dipyrrinones (2−7)[a]

		λ^{max} (ϵ^{max})[b] for						
solvent	ϵ[c]	**1**	**4**	**3**	**2**	**5**	**6**	**7**
C_6H_6	2.3	421 (29 500)	408 (30 500)	406 (24 300)	398 (22 800) 420 (19 000)	398 (30 100) 419 (25 400)	407 (34 400) 429 (32 500)	392 (23 500)
$CHCl_3$	4.7	420 (29 800)	408 (32 400)	407 (24 900)	401 (24 280) 420 (21 000)	406 (33 100) 429 (27 400)	407 (32 800) 429 (28 400)	394 (25 300)
CH_3OH	32.6	413 (34 300)	411 (34 500)	412 (28 300)	403 (31 300) 424 (26 800)	402 (35 800) 425 (30 400)	402 (33 800) 424 (29 300)	396 (27 200)
CH_3CN	36.2	409 (30 500)	400 (33 800)	400 (23 600)	398 (28 000) 419 (24 300)	396 (33 000) 417 (26 500)	396 (31 300) 417 (29 300)	395 (26 700)
$(CD_3)_2SO$	49	410 (34 200)	410 (35 300)	411 (28 300)	405 (33 700) 427 (31 800)	405 (37 600) 428 (35 300)	405 (36 000) 427 (34 200)	395 (26 300)

[a] Data obtained at 22 °C on (2−4) × 10^{-5} M solutions. [b] λ^{max} in nm, ϵ^{max} in M^{-1} cm^{-1}. [c] Dielectric constants: Gordon, A. J.; Ford, R. A. *The Chemist's Companion*; Wiley: New York, 1972; pp 4−8.

Solution and Chromatographic Properties. Hemirubin (**1**) is less soluble in chloroform than bilirubin. It is more polar and is soluble in dilute aqueous bicarbonate, where bilirubin is insoluble. Consistent with these properties, **1** has a shorter retention time (~9.2 min) on reversed-phase HPLC than mesobilirubin-XIIIα (~16.5 min). It also has a longer retention time than its methyl ester **4** (~5.2 min), which is close to that of the *o*-xylyldipyrrinone **3** (~6.0 min), suggesting that the ester is more polar than the acid. This unusual HPLC behavior (ester more polar than the parent acid) parallels that observed for bilirubin and mesobilirubin-XIIIα and their dimethyl esters and is yet a further indication of intramolecular hydrogen bonding in the acids. Such distinctions in HPLC behavior are not evident for the 10-oxohemirubin (**2**) and its methyl ester (**5**), however, which have identical retention times (~4.3 min) that are very similar to that of the (*o*-methylbenzoyl)dipyrrinone **6** (~4.6 min) and comparable to **3** and **4** but not **1**.

On silica gel TLC, **1** travels more rapidly than **3** or **4**, again consistent with reduced polarity due to intramolecular hydrogen bonding. This behavior parallels that of bilirubin relative to its dimethyl ester. Curiously, the 10-oxohemirubin (**2**) behaves on TLC as if it were more polar than its ester (**5**), which would appear to suggest less effective intramolecular hydrogen bonding than in **1**. Taken collectively, the chromatographic behavior of **1** is consistent with a structure where the polar dipyrrinone and carboxylic acid groups are tied together by intramolecular hydrogen bonding (Figure 3), whereas for **2** the data suggest less effective hydrogen bonding of its CO_2H group.

Conclusions

Hemirubin (**1**), a model for one-half bilirubin, has been synthesized and, like bilirubin, is found to adopt a folded, intramolecularly hydrogen-bonded conformation as its energy-minimum structure. As such, it is less polar than its methyl ester and much less polar than bilirubin analogues, e.g., xanthobilirubic acid, that are incapable of intramolecular hydrogen bonding. Whether this analogue behaves similarly to bilirubin in hepatic metabolism, requiring glucuronidation of its hydrogen-bonded carboxylic acid in order to be excreted into bile, remains to be investigated.

Experimental Section

General Methods. All UV−vis and infrared (IR) spectra and nuclear magnetic resonance (NMR) spectra were determined as reported previously.[12,14,16] NMR spectra were obtained at 300 and 500 MHz and reported in δ (ppm). HMQC, HMBC, NOE, and T_1 experiments were carried out on a 500 MHz spectrometer. Melting points are uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ. Flash column chromatography was carried out using Woelm silica gel F, thin-layer chromatography grade. Radial chromatography was carried out on Merck silica gel PF_{254} with gypsum (preparative-layer grade). HPLC analyses were carried out on a Beckman-Altex ultrasphere-IP 5 μm C(18) ODS column (25 × 0.46 cm) and ODS precolumn (4.5 × 0.46 cm). The flow rate was 1.0 mL/min, and the elution solvent was 0.1 M di-*n*-octylamine acetate in 5% aqueous methanol (pH 7.7, 31 °C). All solvents were reagent grade obtained from Aldrich, Acros Organics, or Fisher. Deuterated chloroform and dimethyl sulfoxide were from Cambridge Isotope Laboratories.

Ethyl acetate, 2,4-pentanedione, diisopropropylamine, *n*-butyllithium in hexane, tin tetrachloride, and potassium borohydride were from Aldrich; peracetic acid, 2-toluoyl chloride, sodium hydroxide, thionyl chloride, titanium tetrachloride, and 2-propanol were from Fisher-Acros; hydrochloric acid and glacial acetic acid, were from EM Science, Inc.; nickel−aluminum alloy was from W. R. Grace & Co.; 2-bromotoluene was from J. T. Baker Co.

3-(2-Carboxyphenyl)propenoic acid (11) and **3-(2-carboxyphenyl)propanoic acid (10)**[23] were prepared in 68% and 86% yields, respectively, according to literature procedures.[23]

Methyl 3-(2-Carboxyphenyl)propanoate (9). 3-(2-Carboxyphenyl)propanoic acid (15.4 g, 697 mmol) and methanol (500 mL) were placed in a 1000 mL round-bottom flask. To this mixture was added concentrated sulfuric acid (8 mL) dropwise over a period of 10 min with magnetic stirring. The solution was allowed to stir another 30 min at room temperature; the solvent was then removed (rotovap) at 30−35 °C to 1/10 volume. Water (200 mL) was added, followed by dropwise addition of sodium hydroxide (100 mL, 2 M). With the appropriate rate of addition, the temperature of the reaction mixture rose slowly to 30−35 °C but should not exceed 40 °C. A solid white material began to separate from the solution; when the NaOH addition was complete, the mixture was stirred for 1 h. After being cooled to 0 °C on an ice bath, the solid was collected by suction filtration and washed with water (3 × 100 mL). Air drying gave 14.0 g (85%) of crude product as white crystalline solid, which is sufficiently pure for use in the next step: mp 80−82 °C (lit.[24] mp 83−84 °C); ^{1}H NMR ($(CD_3)_2SO$, 300 MHz) δ 2.56 (t, J = 7.32 Hz, 2H, CH_2CH_2COO), 3.13 (t, J = 7.32 Hz, 2H, CH_2CH_2COO), 3.53 (s, 3H, OCH_3), 7.25−7.35 (m, 2H, 2Ar-*H*), 7.40−7.45 (m, 2H, 2Ar-*H*), 12.91 (brs, 1H, COO*H*) ppm; ^{13}C NMR ($(CD_3)_2SO$): δ 29.27 (CH_2CH_2COO), 35.35 (CH_2CH_2COO), 51.45 (OCH_3), 94.14, 126.6, 130.6, 131.0, 132.0, 141.7, 168.8 (COOH), 172.9 (COOMe) ppm.

(23) Page, G. A.; Tarbell, G. S. *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. IV, pp 136−140.

(24) Arth, C.; Clemens, M.; Muse, W. *Liebigs Ann. Chem.* **1994**, 259−263.

9-(2-Methylbenzoyl)-2,3,7,8-tetramethyldipyrrinone (6). 2-Methylbenzoyl Chloride. 2-Methylbenzoic acid (20 g, 0.147 mol) and thionyl chloride (61 g, 37 mL, 0.51 mol, 3.5 mol equiv) were placed in a 100 mL round-bottom flask. The mixture was allowed to warm to 50 °C (heat by mantle) under protection of a drying tube equipped with anhyd calcium chloride. After all solid material was dissolved, the solution was stirred for 10 min at the same temperature. Excess thionyl chloride was distilled under water aspiration, and the residue was distilled at high vacuum. The desired product was collected at 54−56 °C /1 mmHg to give 20 g (90% yield): bp 54−56°/1 mmHg; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.56 (s, 3H, C*H*$_3$), 7.27−7.37 (m, 2H, 2Ar-*H*), 7.49−7.54 (m, 1H, Ar-*H*), 8.20 (d, J = 7.81 Hz, 1H, Ar-*H*) ppm; ^{13}C NMR ($CDCl_3$) δ 21.67 (*C*H$_3$), 126.2, 131.7, 132.3, 133.6, 133.9, 141.1, 167.2 (*C*OOCl). The compound was used directly in the next step.

Condensation. 2-Methylbenzoyl chloride (50 mg, 0.32 mmol, 6.9 mol equiv) and dry dichloromethane (50 mL) were added to a 100 mL three-neck flask fitted with a magnetic stirrer and nitrogen inlet. The flask was cooled with an ice−salt bath, and nitrogen was bubbled through the solution. After 20 min, tin tetrachloride (three drops) was introduced, followed by addition 2,3,7,8-tetramethyldipyrrinone (**7**) (10 mg, 0.046 mol). The mixture was stirred for another 20 min after the addition was complete at 0 °C. Before it was poured into ice, the mixture was allowed to stir for 1 h at room temperature. The aqueous mixture was extracted with dichloromethane (3 × 50 mL), and the combined extract was washed with hydrochloric acid (2 × 30 mL, 10%) followed by water (2 × 30 mL) and saturated aqueous sodium bicarbonate (50 mL). The organic extracts were dried over anhyd sodium sulfate, the solvent was removed, and the residue was purified by flash chromatography using dichloromethane−methanol (97/3 by vol) as eluant to yield the desired compound. After recrystallization from methanol, the desired product was obtained (10.8 mg 70% yield): mp 302−304 °C; IR (film): ν 3450, 3343, 2843, 1660, 1617, 1438, 1406, 1385, 1273, 1169 cm^{-1}; UV−vis (Table 4); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.61 (s, 3H, C*H*$_3$), 1.95 (s, 3H, C*H*$_3$), 2.02 (s, 3H, C*H*$_3$), 2.11 (s, 3H, C*H*$_3$), 2.31 (s, 3H, C*H*$_3$), 5.95 (s, 1H, −C*H*═), 7.25−7.34 (m, 4H, 4 Ar-*H*), 8.65 (br s, 1H, N*H*), 9.40 (br s, 1H, N*H*) ppm; ^{1}H NMR (90% $CDCl_3$ and 10% $(CD_3)_2SO$, 300 MHz) δ 1.28 (s, 3H, C*H*$_3$), 1.44 (s, 3H, C*H*$_3$), 1.52 (s, 3H, C*H*$_3$), 1.70 (s, 3H, C*H*$_3$), 2.66 (s, 3H, C*H*$_3$), 5.31 (s, 1H, −C*H*═), 6.64−6.75 (m, 4H, 4 Ar-*H*), 10.04 (br s, 1H, N*H*), 10.44 (br s, 1H, N*H*) ppm; ^{1}H NMR ($(CD_3)_2SO$, 300 MHz): δ 1.49 (s, 3H, C*H*$_3$), 1.77 (s, 3H, C*H*$_3$), 1.96 (s, 3H, C*H*$_3$), 2.05 (s, 3H, C*H*$_3$), 2.18 (s, 3H, C*H*$_3$), 5.93 (s, 1H, −C*H*═), 7.18−7.36 (m, 4H, 4 Ar-*H*), 10.50 (br s, 1H, N*H*), 11.13 (br s, 1H, N*H*) ppm; ^{13}C NMR (90% $CDCl_3$ and 10% $(CD_3)_2SO$) δ 8.01 (*C*H$_3$), 8.89 (*C*H$_3$), 9.46 (*C*H$_3$), 10.45 (*C*H$_3$), 18.43 (*C*H$_3$), 94.84, 123.2, 124.3, 125.1, 126.3, 126.7, 127.4, 127.5, 128.7, 130.0, 130.2, 134.3, 135.4, 141.1, 172.8 (C═O, lactam), 185.9 (*C*═O) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ 8.57 (*C*H$_3$), 9.35 (*C*H$_3$), 9.78 (*C*H$_3$), 10.49 (*C*H$_3$), 18.95 (*C*H$_3$), 95.78, 123.6, 126.1, 127.0, 127.3, 127.8, 129.7, 130.8, 130.9, 134.7, 135.0, 136.4, 141.3, 142.2, 173.0 (C═O, lactam), 186.2 (*C*═O) ppm; MS(FAB) m/z = 334 (M^+).

Anal. Calcd for $C_{21}H_{22}N_2O_2$ (334.4): C, 75.45; H, 6.59; N, 8.38. Found: C, 75.20; H, 6.33; N, 8.06.

9-(2-Methylbenzyl)-2,3,7,8-tetramethyldipyrrinone (3). Method A: Condensation of Dipyrrinone with 2-(Bromomethyl)toluene (5). To a mixture of 2,3,7,8-tetramethyldipyrrinone (10 mg, 46 mmol) and 2-(bromo)methyltoluene (100 mg, 540 mmol, 11.7 mol equiv) in methanol (10 mL) was added *p*-toluenesulfonic acid monohydrate (10 mg, 58 mmol, 1.26 mol equiv). The solution was stirred under nitrogen protection for 12 h at 40 °C. After being cooled to room temperature, the mixture was kept for 5 h at −20 °C, and the resulting precipitate was collected by suction titration. The solid was crystallized from dichloromethane−hexane to yield 10.9 mg of **5** (73% yield).

Method B: Reduction of 6. 9-(2-Methylbenzoyl)-2,3,7,8-tetramethyldipyrrinone (**6**) (50 mg, 0.15 mmol) and *2*-propanol (50 mL) were placed in a 100 mL flask. The mixture was heated to reflux under nitrogen; potassium borohydride (200 mg, 3.7 mmol, 24 mol equiv) was then added in small portions over 20 min. The mixture was allowed to reflux for another 2 h after the addition was complete. The reaction was then cooled to room temperature and poured into ice. The mixture was extracted with dichloromethane (3 × 50 mL), and the extract was washed with aqueous hydrochloric acid (2 × 50 mL, 10%) and then water (50 mL). The organic extract was dried over anhyd sodium sulfate and evaporated (rotovap), to give a residue that was crystallized from methanol to yield the desired product (33.5 mg, 70%): mp 278−280 °C; IR (film) ν 3336, 2919, 2860, 1657, 1637, 1458, 1387, 1273, 1182, 938, 732, 691 cm^{-1}; UV−vis (Table 4); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.80 (s, 3H, CH_3), 1.95 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 4.09 (s, 2H, CH_2), 6.80 (s, 1H, −CH═), 6.94−7.57 (m, 4H, phenyl-H), 10.01 (brs, 1H, NH), 10.90 (brs, 1H, NH) ppm; ^{1}H NMR ($(CD_3)_2SO$, 300 MHz) δ 1.72 (s, 3H, CH_3), 1.75 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 3.82 (s, 2H, CH_2), 5.94 (s, 1H, −CH═), 6.70−6.72 (m, 1H, phenyl-H), 7.03−7.13 (m, 2H, 2 phenyl-H), 7.14 (m, 1H, phenyl-H), 9.73 (brs, 1H, NH), 10.29 (brs, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 7.26 (*C*H$_3$), 8.76 (*C*H$_3$), 9.88 (*C*H$_3$), 9.98 (*C*H$_3$), 19.48 (*C*H$_3$), 30.52 (*C*H$_2$), 96.07, 116.6, 120.9, 123.1, 124.1, 125.6, 125.9, 126.4, 128.4, 128.7, 130.0, 135.5, 136.2, 143.8, 173.2 (C═O) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ 8.13 (*C*H$_3$), 9.42 (*C*H$_3$), 10.26 (*C*H$_3$), 10.33 (*C*H$_3$), 19.50 (*C*H$_3$), 29.43 (*C*H$_2$), 95.65, 125.9, 126.1, 126.2, 127.9, 128, 123.0, 130.0, 131.2, 134.3, 135.0, 135.4, 135.8, 141.8, 173.2 (C═O) ppm; MS(FAB) m/z 320 (M^+).

Anal. Calcd for $C_{21}H_{24}N_2O$ (320.4): C, 78.75; H, 7.50; N, 8.75.

Anal. Calcd for $C_{21}H_{24}N_2O\cdot0.5H_2O$ (329.4): C, 76.49; H, 7.58; N, 8.49. Found: C, 76.03; H, 7.30; N 8.03.

9-[2-[2-(Methoxycarbonyl)ethyl]benzoyl]-2,3,7,8-tetramethyldipyrrinone (5). The acid chloride of **10** was first prepared and then condensed with dipyrrinone **7**.

Methyl 3-(2-Carboxyphenyl)propanoyl Chloride (9). Methyl 3-(2-carboxyphenyl)propanoate (5.0 g, 24 mmol) and thionyl chloride (10 g, 6.3 mL, 84 mmol, 3.5 mol equiv) were placed in a 100 mL round-bottom flask. The mixture was equipped with a $CaCl_2$ drying tube and allowed to warm to 50 °C using a heating mantle. After all of the solid was dissolved, the solution was stirred for 10 min at the same temperature. Excess thionyl chloride was removed by distillation under vacuum (water aspirator). The residue was distilled at high vacuum. The desired product (3.4 g, 66% yield) was collected at 120 °C /1 mmHg and used directly in the next step: bp 120 °C/1 mmHg; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.60 (t, J = 7.82 Hz, 2H, CH_2CH_2COO), 3.18 (t, J = 7.82 Hz, 2H, CH_2CH_2COO), 3.61 (s, 3H, OCH_3), 7.31−7.38 (m, 2H, 2Ar-*H*), 7.50−7.55 (m, 1H, Ar-*H*), 8.17 (d, J = 8.30 Hz, 1H, Ar-*H*) ppm; ^{13}C NMR ($CDCl_3$) δ 29.45 (CH_2CH_2COO), 34.61 (CH_2CH_2COO), 51.38 (OCH_3), 126.9, 126.9, 131.2, 133.7, 134.2, 143.0, 167.3 (*C*OCl), 172.6 (*C*OOMe) ppm.

2-[2-(Methoxycarbonyl)ethyl]benzoyl chloride (596 mg, 2.78 mmol, 2.0 mol equiv) and dry dichloromethane (50 mL) were added to a 100 mL three-neck flask fitted with a magnetic stirrer and nitrogen inlet. The flask was cooled with an ice−salt bath, and nitrogen was bubbled throughout the solution. After 20 min, 2,3,7,8-tetramethyldipyrrinone (**7**) (300 mg, 1.39 mmol mol) was added, followed by tin tetrachloride (3.61 g, 1.62 mL, 13.9 mmol, 10 mol equiv). The mixture was stirred for another 20 min at 0 °C after the addition was complete. Before it was poured into an ice−HCl solution (100 g of ice + 50 mL of concentrated hydrochloric acid), the mixture was allowed to stir for 2 h at room temperature. The aqueous mixture was extracted with dichloromethane (3 × 50 mL). The combined extract was washed with hydrochloric acid (2 × 30 mL, 10%), water (2 × 30 mL), and saturated aqueous sodium bicarbonate (50 mL). The organic extract was dried over anhyd sodium sulfate, the solvent was removed, and the residue was purified by flash chromatographic column using dichloromethane−methanol (97/3, by vol) as eluant to yield the desired compound, which was further purified by recrystallization from methanol. Pure title product was obtained (545 mg, 95%): mp 212−214 °C; IR (film) ν 3336, 2835, 2830,

1736, 1672, 1617, 1560, 1508, 1490, 1432, 1405, 1166, 934, 757 cm^{-1}; UV−Vis (Table 4); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.69 (s, 3H, CH_3), 1.94 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.63 (t, J = 7.32 Hz, 2H, CH_2CH_2COO), 3.00 (t, J = 7.32 Hz, 2H, CH_2CH_2COO), 3.62 (s, 3H, OCH_3), 5.95 (s, 1H, −C*H*═), 7.26−7.38 (m, 4H, 4 Ar-*H*), 9.36 (br s, 1H, N*H*), 9.57 (br s, 1H, N*H*) ppm; ^1H NMR ($(CD_3)_2SO$, 300 MHz) δ 1.47 (s, 3H, CH_3), 1.75 (s, 3H, CH_3), 1.95 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.53 (t, J = 7.32 Hz, 2H, CH_2CH_2COO), 2.78 (t, J = 7.32 Hz, 2H, CH_2CH_2COO), 3.49 (s, 3H, OCH_3), 5.91 (s, 1H, −C*H*═), 7.19−7.38 (m, 4H, 4 Ar-*H*), 10.46 (br s, 1H, N*H*), 11.13 (br s, 1H, N*H*) ppm; ^{13}C NMR ($CDCl_3$) δ 8.34 (CH_3), 9.09 (CH_3), 9.59 (CH_3), 10.56 (CH_3), 28.01 (CH_2CH_2COO), 35.19 (CH_2CH_2COO), 51.40 (OCH_3), 96.21, 126.1, 127.3, 128.6, 128.8, 129.6, 129.7, 130.5, 130.7, 130.7, 137.0, 138.0, 140.1, 141.5, 173.2 (C═O, lactam), 173.5 (*C*OOMe), 186.3 (*C*═O) ppm; ^{13}C NMR ($(CD_3)_2SO$) (Table 2); MS(FAB) *m*/*z* 406 (M^+).

Anal. Calcd for $C_{24}H_{26}N_2O_4$ (406.5): C, 70.93; H, 6.40; N, 6.89. Found: C, 70.50; H, 6.39; N, 6.89.

9-[2-(2-Carboxyethyl)benzoyl]-2,3,7,8-tetramethyldipyrrinone (2). 9-[2-(2-Methoxycarbonyl)benzoyl]-2,3,7,8-tetramethyldipyrrinone (**5**) (50 mg, 0.123 mmol) was dissolved in a solution of methanol (10 mL) and aqueous sodium hydroxide (10%, 3 mL). The mixture was stirred and warmed to reflux under nitrogen atmosphere protection for 2 h. After being cooled to room temperature, the mixture was brought to pH 7 by adding acetic acid dropwise. The solution was extracted with dichloromethane (3 × 50 mL), and the combined extracts were washed with water (2 × 50 mL). The organic extract was dried over anhyd sodium sulfate, the solvent was removed (rotovap), and the residue was crystallized from methanol to yield the desired product (36.2 mg) in 75% yield: mp 200−202 °C; IR (film) ν 3376, 2840, 1690, 1520, 1490, 1430, 1405, 1350, 1268, 1166, 933, 836, 757, 631 cm^{-1}; UV−vis (Table 4); ^1H NMR ($CDCl_3$, 500 MHz) δ 1.85 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.83 (t, J = 6.50 Hz, 2H, CH_2CH_2COO), 3.08 (t, J = 6.50 Hz, 2H, CH_2CH_2COO), 6.05 (s, 1H, −C*H*═), 7.15−7.17 (m, 2H, 2 Ar-H), 7.40 (m, 2H, 2 Ar-*H*), 8.15 (s, 1H, N*H*), 10.69 (s, 1H, N*H*), 12.37 (br s, 1H, COO*H*) ppm; ^1H NMR ($(CD_3)_2SO$, 500 MHz) δ 1.51 (s, 3H, CH_3), 1.79 (s, 3H, CH_3), 1.98 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.46 (t, J = 7.50 Hz, 2H, CH_2CH_2COO), 2.77 (t, J = 7.50 Hz, 2H, CH_2CH_2COO), 5.95 (s, 1H, −C*H*═), 7.23 (d, J = 7.50 Hz, 1H, Ar-H), 7.30 (t, J = 7.50, 7.0 Hz, 1H, Ar-H), 7.38 (d, J = 7.50 Hz, 1H, Ar-H), 7.41 (t, J = 7.50, 7.0 Hz, 1H, Ar-H), 10.50 (s, 1H, N*H*), 11.17 (s, 1H, N*H*), 12.10 (s, 1H, COO*H*) ppm; ^{13}C NMR ($CDCl_3$) δ 8.02 (CH_3), 8.70 (CH_3), 9.49 (CH_3), 10.33 (CH_3), 25.89 (CH_2CH_2COO), 34.21 (CH_2CH_2COO), 98.74, 125.3, 125.3, 126.9, 127.8, 128.2, 129.0, 129.0, 129.2, 129.8, 129.9, 137.6, 139.4, 141.4, 173.3 (C═O, lactam), 175.5 (*C*OOH), 177.6 (*C*═O) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ 8.59 (CH_3), 9.36 (CH_3), 9.78 (CH_3), 10.78 (CH_3), 27.98 (CH_2CH_2COO), 35.31 (CH_2CH_2COO), 95.71, 123.7, 126.5, 127.3, 128.0, 129.8, 130.3, 130.8, 130.9, 131.2, 136.5, 138.2, 141.0, 142.1, 173.0 (C═O, lactam), 173.7 (*C*OOH), 186.0 (*C*═O) ppm; MS(FAB) *m*/*z* 392 (M^+).

Anal. Calcd for $C_{23}H_{24}N_2O_4$ (392.5): C, 70.39; H 6.16; N, 7.14. Anal. Calcd for $C_{23}H_{24}N_2O_4 \cdot CH_3OH$ (423): C, 68.09; H, 6.38; N, 6.66. Found: C, 68.50; H, 6.09; N, 6.66.

9-[2-(2-Carboxyethyl)benzyl]-2,3,7,8-tetramethyldipyrrinone (1, Hemirubin). 9-[2-[2-(Methoxycarbonyl)ethyl]benzoyl]-2,3,7,8-tetramethyldipyrrinone (**5**) (115 mg, 0.28 mmol) and *2*-propanol (50 mL) were placed in a 100 mL flask. The mixture was heated to reflux under nitrogen; then potassium borohydride (200 mg, 3.70 mmol, 13.27 mol equiv) was added in small portions over 20 min. The mixture was allowed to heat at reflux for another 2 h after the addition was complete. The reaction was cooled to room temperature and poured onto ice. The mixture was extracted with dichloromethane (3 × 50 mL), and the extract was washed with aqueous hydrochloric acid (2 × 50 mL, 10%) and water (50 mL). The organic layer was dried over anhyd sodium sulfate, and the solvent was removed (rotovap). The residue was crystallized from methanol to yield the desired product (67 mg) in 63% yield: mp 220° dec; IR (film) ν 3425, 3340, 2916, 2863, 1163, 1443, 1355, 1273, 1179, 940, 756, 731, 695 cm^{-1}; UV−vis (Table 4); ^1H NMR ($CDCl_3$, 500 MHz) δ 1.84 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 2.98 (t, J = 5.5 Hz, 2H, CH_2CH_2COO), 3.07 (t, J = 5.50 Hz, 2H, CH_2CH_2COO), 4.08 (s, 2H, −CH_2−), 6.07 (s, 1H, −C*H*═), 6.98 (d, J = 7.5 Hz, 1H, Ar-*H*), 7.05 (t, J = 7.5 Hz, 1H, Ar-*H*), 7.13 (t, J = 8.0 Hz, 1H, Ar-*H*), 7.20 (d, J = 7.5 Hz, 1H, Ar-*H*), 8.98 (s, 1H, N*H*), 10.53 (s, 1H, N*H*), 12.61 (br s, 1H, COO*H*) ppm; ^1H NMR ($(CD_3)_2SO$, 500 MHz) δ 1.76 (s, 3H, CH_3), 1.79 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.47 (t, J = 7.50 Hz, 2H, CH_2CH_2COO), 2.90 (t, J = 7.50 Hz, 2H, CH_2CH_2COO), 3.92 (s, 2H, CH_2) 5.95 (s, 1H, −C*H*═), 6.78 (d, J = 7.50 Hz, 1H, Ar−H), 7.06−7.14 (m, 2H, 2 Ar-H), 7.16 (d, J = 8.00 Hz, 1H, Ar-H), 9.72 (s, 1H, N*H*), 10.28 (s, 1H, N*H*), 12.21 (s, 1H, COO*H*) ppm; ^{13}C NMR ($CDCl_3$) δ 7.91 (CH_3), 8.86 (CH_3), 9.41 (CH_3), 9.47 (CH_3), 24.58 (CH_2CH_2COO), 27.01 (CH_2), 34.80 (CH_2CH_2COO), 101.0, 126.1, 126.2, 128.6, 128.6, 129.3, 129.2, 130.8, 135.4, 138.0, 138.4, 148.7, 153.1, 153.7, 174.5 (C═O, lactam), 178.9 (*C*OOH) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ 8.20 (CH_3), 8.73 (CH_3), 9.33 (CH_3), 9.46 (CH_3), 27.24 (CH_2CH_2COO), 28.45 (CH_2), 34.04 (CH_2CH_2COO), 97.73, 116.1, 122.3, 122.7, 123.5, 126.0, 126.0, 126.0, 128.4, 128.9, 130.6, 137.5, 138.4, 141.4, 171.8 (C═O, lactam), 173.7 (*C*OOH) ppm; MS(FAB) *m*/*z* 378 (M^+).

Anal. Calcd for $C_{23}H_{26}N_2O_3$ (378.5): C, 73.01; H, 6.88; N, 7.41. Found: C, 73.16; H, 6.92, N, 7.40.

9-[2-[2-(Methoxycarbonyl)ethyl]benzyl]-2,3,7,8-tetramethyldipyrrinone (4). 9-[2-(2-Carboxyethyl)benzyl]-2,3,7,8-tetramethyldipyrrinone (**1**) (15 mg, 39.68 mmol) was dissolved in dry methanol (50 mL). To this mixture was added concentrated sulfuric acid (1 mL) dropwise over 5 min. The solution was stirred at room temperature for 1 h before dichloromethane (100 mL) and water (100 mL) were added. The organic layer was separated, and the aqueous phase was extracted with dichloromethane (2 × 50 mL). The combined organic extract was successively washed with water (2 × 70 mL), saturated aqueous sodium bicarbonate (70 mL), and brine (70 mL). The organic extract was dried over anhyd sodium sulfate, the solvent was removed (rotovap), and the residue was purified by radial chromatography using 2% methanol in dichloromethane as eluant. The main yellow fraction was collected and recrystallized from methanol and then from dichloromethane-hexane to afford the expected product (12 mg) in 77% yield: mp 236−238 °C dec; IR (KBr, film) ν 3344, 2923, 2854, 1741, 1735, 1662, 1636, 1362, 1276, 1176, 934, 756, 691 cm^{-1}; UV−vis (Table 4); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.65 (s, 3H, CH_3), 1.91 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.54 (t, J = 7.32 Hz, 2H, CH_2CH_2COO), 3.04 (t, J = 7.32 Hz, 2H, CH_2CH_2COO), 3.66 (s, 3H, OCH_3), 4.16 (s, 2H, CH_2), 6.11 (s, 1H, −C*H*═), 7.00−7.14 (m, 4H, 4 Ar-H), 10.15 (s, 1H, N*H*), 10.79 (s, 1H, N*H*) ppm; ^1H NMR ($(CD_3)_2SO$, 300 MHz) δ 1.74 (s, 6H, 2 CH_3), 2.01 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.49 (t, J = 7.32 Hz, 2H, CH_2CH_2COO), 2.90 (t, J = 7.32 Hz, 2H, CH_2CH_2COO), 3.55 (s, 3H, OCH_3), 3.90 (s, 2H, −CH_2−), 5.93 (s, 1H, −C*H*═), 6.77 (d, J = 6.35 Hz, 1H, Ar-*H*), 7.08−7.12 (m, 3H, 3 Ar-H), 9.71 (s, 1H, N*H*), 10.26 (s, 1H, N*H*) ppm; ^{13}C NMR ($CDCl_3$) δ 7.99 (CH_3), 8.02 (CH_3), 9.41 (CH_3), 9.61 (CH_3), 27.65 (CH_2CH_2COO), 29.63 (CH_2), 34.43 (CH_2CH_2COO), 51.43 (OCH_3), 100.5, 116.9, 122.5, 123.6, 124.5, 126.3, 126.4, 128.5, 128.9, 129.1, 132.3, 137.1, 138.0, 141.9, 173.6 (C═O, lactam, and *C*OO) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ 8.53 (CH_3), 9.06 (CH_3), 9.64 (CH_3), 9.78 (CH_3), 27.54 (CH_2CH_2COO), 29.00 (CH_2), 34.11 (CH_2CH_2COO), 51.51 (OCH_3), 98.04, 122.6, 123.0, 125.4, 125.5, 126.1, 126.5, 126.6, 128.9, 129.0, 129.3, 137.9, 138.5, 141.7, 172.1 (C═O, lactam, and *C*OO) ppm; MS(FAB) *m*/*z* 392 [M^+].

Anal. Calcd for $C_{24}H_{28}N_2O_3$ (392.5): C, 73.47; H, 7.14; N, 7.14. Found: C, 73.35; H, 7.19; N, 7.14.

Acknowledgment. We thank the National Institutes of Health (HD-17779) for support of this research and the National Science Foundation (CHE-9214294 and DIR-9102893) for funds to purchase the 500 MHz NMR spectrometer and mass spectrometer used in this study. We are indebted to Mr. Michael Huggins for determining the energy-minimum structures of **1** and **2** and for creating their ball and stick displays.

Supporting Information Available: Spectra for compounds **1**−**7** in $(CD_3)_2SO$ solvent are reported herein (14 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead for ordering information.

JO972227R