Synthesis and Properties of Bilirubin Analogs with One Propionic Acid Group

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Unsymmetric bile pigment analogs of mesobilirubin-XIIIα and mesobilirubin-IVα were synthesized following oxidative coupling of dipyrrinones: methyl xanthobilirubinate with kryptopyrromethenone; methyl ψ-xanthobilirubinate with kryptopyrromethenone.

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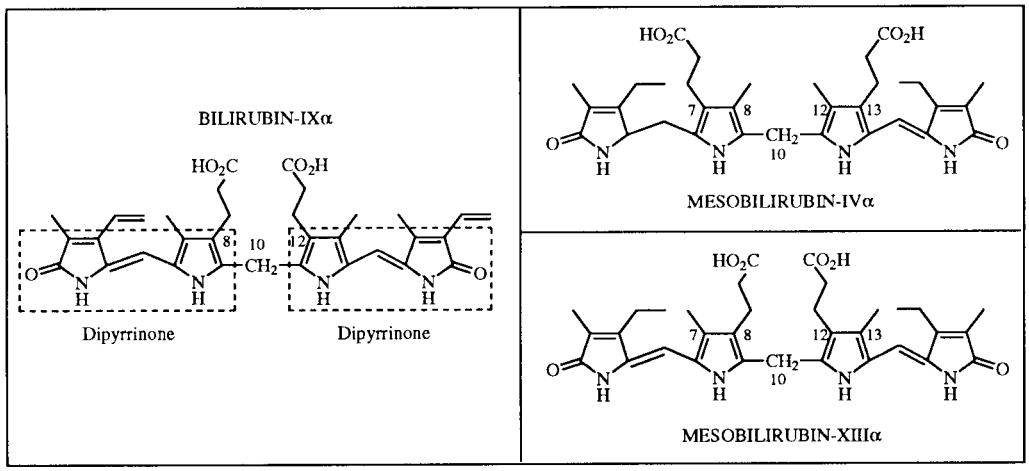
Introduction.

Bilirubin-IXα, the cytotoxic yellow pigment of jaundice, is composed of two dipyrrinone chromophores linked at their α-carbons by a -CH_2- group. The ring β-positions of each dipyrrinone are substituted by vinyl, methyl and propionic acid groups. By varying the order of the β-substituents, a large array of bilirubin analogs becomes possible, although only one (bilirubin itself) is the major pigment produced and eliminated in mammals (at the rate of ~300 mg/day/individual—from the breakdown of ~10^{11} red blood cells per day) [1-3]. The location of the propionic acid groups has a profound influence on the spectroscopic, solution and metabolic properties of bilirubin [1,4]. When the propionic acid groups are moved away from their natural positions at C(8) and C(12), the resulting new pigments (such as mesobilirubin-IVα) are much more polar, much less soluble in solvents such as chloroform or benzene (in which the uv-visible λ max are blue shifted), more soluble in weak aqueous base, and more excretable across the liver into bile [4,5]. On the other hand, when the vinyl groups are reduced to ethyl or replaced by methyl, as in mesobilirubin-XIIIα, these properties of the pigment remain relatively unchanged.

dipyrrinones are rotated about the C(10) –CH_2– so as to bring the CO_2H and the dipyrrinone pyrrole NH and lactam -NH-C=O groups into sufficiently close proximity for intramolecular hydrogen bonding (Figure 1A) [6-8]. With tetrahedral hybridization at C(10), the molecule is bent across the middle to form either of two enantiomeric conformers shaped like ridge-tiles [9a] (Figure 1B) and seen in equal numbers in crystals of bilirubin and its dicarboxylate salts—to the exclusion of other conformations [9]. In the crystalline state, bilirubin molecules can stretch and vibrate but are otherwise restrained. However, when dissolved in organic solvents, bilirubin is potentially more flexible and might be expected to exhibit more conformational freedom, forming hydrogen bonds to solvent molecules, to other bilirubin molecules, or intramolecularly as it does in the crystal [10,11]. Despite a multitude of conformational possibilities, it now seems clear that intramolecularly hydrogen-bonded structures like those in Figure 1 prevail in non-polar solvents such as chloroform, in polar solvents such as acetone and ethanol, and even in water at high *p*H (where bilirubin is present as the dicarboxylate dianion) [12].

In order to explore the importance and location of a single



The unique role of the propionic acid groups at C(8) and C(12) is stabilization of a conformation where the propionic acid in stabilizing the ridge-tile conformation, we synthesized and determined the properties of: (i) the

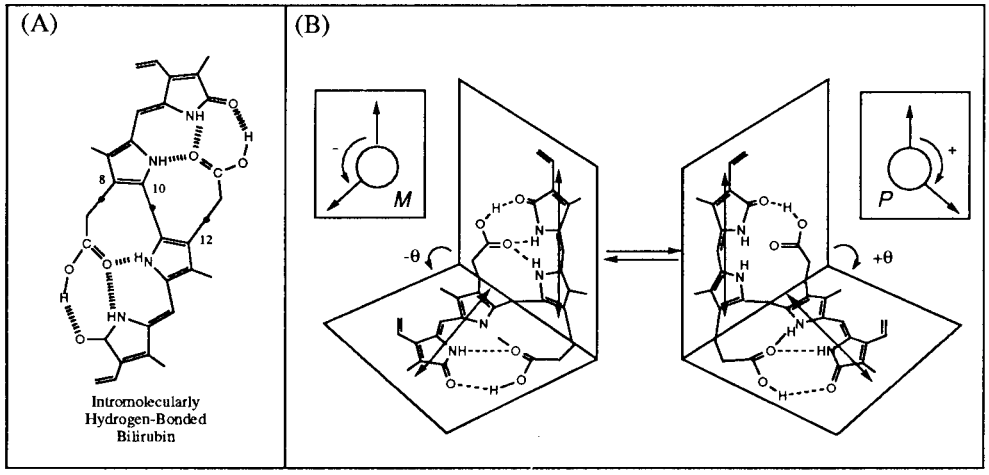


Figure 1. (A) Planar representation of bilirubin with dipyrrinone groups rotated about C(10) so as to enable effective intramolecular hydrogen bonding between the propionic acid CO_2H groups and the pyrrole NH and lactam -NH-C=O groups. (B) Interconverting intramolecularly hydrogen-bonded enantiomeric conformers of bilirubin. The double headed arrows represent the dipyrrinone long wavelength electric transition moment vectors (dipoles) associated with the uv-visible spectrum of the pigment. The relative helicities (*M*, minus or *P*, plus) of the vectors are shown (inset) for each enantiomer. The dihedral angle (θ) at the intersection of the planes containing each of the two dipyrrinones is ~100°.

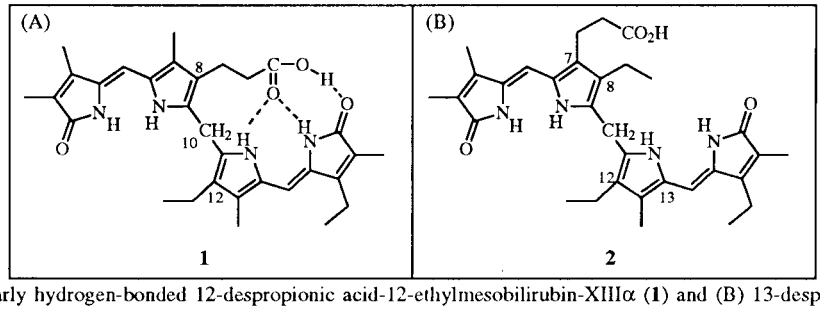


Figure 2. (A) Intramolecularly hydrogen-bonded 12-despropionic acid-12-ethylmesobilirubin-XIIIα (**1**) and (B) 13-despropionic acid-13-methyl-12-desmethyl-12-ethylmesobilirubin-IVα (**2**) absent intramolecular hydrogen bonds.

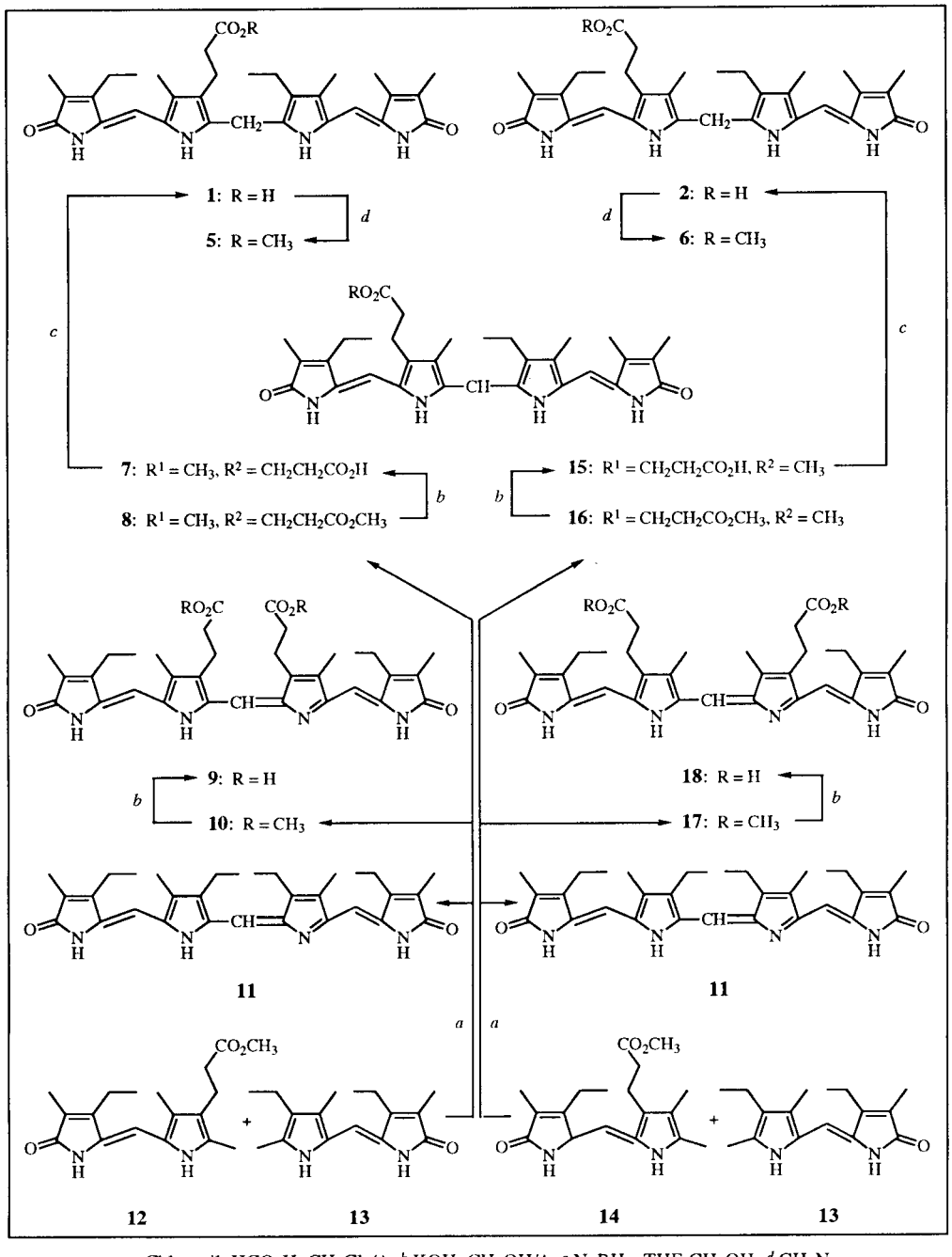
mesobilirubin-XIIIα (**3**) analog, 12-despropionic acid-12-ethylmesobilirubin-XIIIα (**1**), and (ii) the mesobilirubin-IVα (**4**) analog, 13-despropionic acid-13-methyl-12-desmethyl-12-ethylmesobilirubin-IVα (**2**) (Figure 2). Their conformations are analyzed by ^{1}H-nmr and uv-visible spectroscopy. The work is important because it shows that even one propionic acid group has a unique ability to stabilize pigment conformation.

Synthesis.

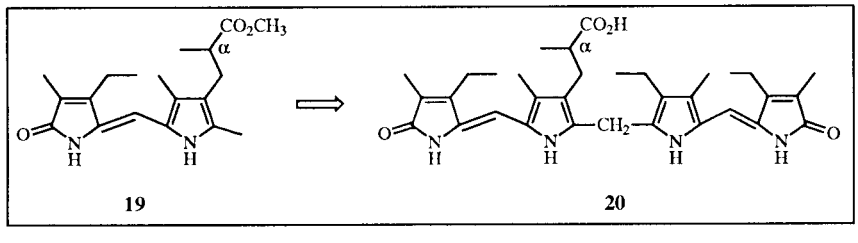
Our initial approach toward the syntheses of **1** and **2** focused on the acid-catalyzed "reverse scrambling" reaction [13,14,15]. From a 50:50 mixture of mesobilirubin-XIIIα and etiobilirubin-IVγ one would obtain 50% of **1** in a mixture of all three [14]. And from a mixture of mesobilirubin-IVα and etiobilirubin-IVγ one would obtain 50% of **2** in a mixture of all three. Although the scrambling reaction proceeded smoothly, separation of the desired monopropionic acid rubins proved surprisingly difficult, and so an alternative synthetic method was pursued. From our earlier work, we knew that oxidative cross coupling of dipyrrinones affored a verdin mixture; however, this avenue for preparing unsymmetric rubins or verdins was not pursued because we found verdin ester mixtures difficult to separate. In the current work we learned that verdin acids can often be separated easily, affording an attractive alternative to the synthesis of unsymmetric rubins *via* the "reverse scrambling" reaction [13]. Thus, as shown in Scheme 1, reaction of methyl xanthobilirubinate (**12**) with kryptopyrromethenone (**13**) gave the symmetric mesobiliverdin-XIIIα dimethyl ester (**10**) and etiobiliverdin-IVγ (**11**), along with ~50% of the desired unsymmetric verdin (**8**). Separation of this mixture proved difficult; however, after saponfication of the esters to the verdin acids, the mixture could be separated easily using extraction and chromatographic methods to afford pure verdins **7**, **9** and **11**. The unsymmetric verdin (**7**) was reduced smoothly with sodium borohydride to the desired unsymmetric rubin (**1**).

Similarly, oxidative cross coupling of methyl ψ–xanthobilirubinate (**14**) with kryptopyrromethenone (**13**) afforded the mixture of verdins **16**, **17** and **11**. And as above, although the verdin ester mixture proved difficult to separate, the verdin acid mixture was relatively easy to

Scheme 1



[a] Chloranil, HCO_2H, CH_2Cl_2/Δ; [b] KOH, CH_3OH/Δ; [c] $NaBH_4$, THF-CH_3OH; [d] CH_2N_2.



separate by extraction and chromatography. The separated unsymmetric verdin (**15**) was converted to the unsymmetric rubin (**2**) using sodium borohydride. Although we could not anticipate beforehand the considerable ease of

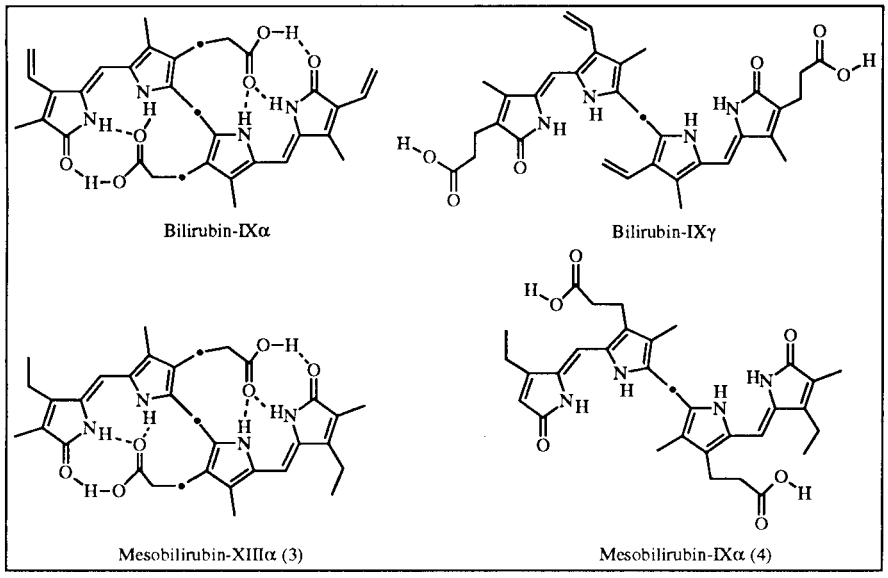


Figure 3. Conformational structures of bilirubin-IXα, mesobilirubin-XIIIα, bilirubin-IXγ and mesobilirubin-IVα illustrating their ability to engage in intramolecular hydrogen bonding between the propionic acid and dipyrrinone moieties.

separation of the verdin acids here and above, synthesis and separation of the verdins proved easier than separation of the rubins. The facility of the synthetic approach described above was further illustrated by the synthesis of **20**, an analog of **1** with an α–methyl group in the propionic acid chain. It was conveniently prepared by oxidative coupling of kryptopyrromethenone **13** with the known [12] dipyrrinone **19**.

Properties.

The properties of bilirubins are now known to depend considerably on the ability of the pigment's polar groups to interact through intramolecular hydrogen bonding. Thus, sequestration of the propionic acid carboxyl groups of bilirubin-IXα and its symmetric analog mesobilirubin-XIIIα through intramolecular hydrogen bonding with the dipyrrinone lactam C=O and N-H groups and the pyrrole N-H makes these pigments much less polar and more lipophilic than their counterparts with propionic acid groups anchored to other ring carbons, as in bilirubin-IXγ and mesobilirubin-IVα (Figure 3). For example, bilirubin-IXγ and mesobilirubin-IVα, in which the COOH groups cannot or do not participate in intramolecular hydrogen bonding, can be extracted from chloroform into 5% aqueous sodium bicarbonate, but bilirubin-IXα and mesobilirubin XIIIα cannot, and the former pair run faster on reverse phase hplc and more slowly on silica tlc adsorption chromatography than the latter.

The relative polarities of pigments can often be detected and assessed by their chromatographic behavior [16]. Thus, it was interesting to note that mesobilirubin-IVα (**4**) had a significantly shorter retention time (7.2 minutes) than mesobilirubin-XIIIα (**3**) (18.9 minutes) on reverse phase analytical hplc using an eluent (0.1 *M* di-*n*-octylamine acetate in methanol containing 5% water, 1.00 ml/minute flow rate). In reverse phase chromatography, more polar compounds elute faster than less polar. The hplc system used here is known to distinguish between those bile pigments which adopt intramolecular hydrogen bonding (as do bilirubin-IXα and mesobilirubin-XIIIα) and become lipid soluble and those that can't (as in mesobilirubin-IVα and bilirubin glucuronides) and are more polar and more hydrophilic [5,15].

As might be expected, **1** ran faster (11.1 minutes) on reverse phase hplc than its parent, mesobilirubin-XIIIα (**3**) (18.9 minutes) and slower than mesobilirubin-IVα (**4**) (7.2 minutes). Surprisingly, **2** ran faster (9.2 minutes) than **4** and only slightly slower than **1**. Interestingly, **20** ran somewhat slower (14.6 minutes) than **1**, but this may be

Table 1

Solvent Dependence of COO*H* Lactam and Pyrrole N-*H* Chemical Shifts [a]

Pigment	$(CD_3)_2SO$ Lactam	$(CD_3)_2SO$ Pyrrole	$CDCl_3$ Lactam	$CDCl_3$ Pyrrole
1	9.82	10.34	10.53	9.00
	9.78	10.29	8.26	7.93
2	9.82	10.35	10.23	9.92
	9.80	10.35	9.31	8.35
20	9.80	10.30	10.57	8.85
	9.80	10.23	8.00	7.90
Bilirubin-IXα	10.07	10.48	10.80	9.27
	9.93	10.48	10.70	9.30
Mesobilirubin-XIIIα	9.72	10.27	10.57	9.15
Etiobilirubin-IVγ	9.78	10.28	10.58	10.28
Mesobilirubin-XIIIα Dimethyl Ester	9.74	10.40	10.54	10.27

[a] Typically run on 10^{-2} *M* dimethyl sulfoxide-d_6 and 10^{-3} *M* $CDCl_3$ solutions at 21°C.

attributed to the presence of the α-methyl group. The data suggest that both **1** and **20** are less polar than **2**, but all are less polar than **4** and might exhibit significant intramolecular hydrogen bonding. The hplc data are supported qualitatively by the tlc behavior of **1** ($R_f \simeq 0.69$), **2** ($R_f \simeq 0.58$) and **20** ($R_f \simeq 0.50$), as compared with mesobilirubin-XIIIα ($R_f \simeq 0.9$) and mesobilirubin-IVα or even etiobilirubin-IVγ (both $R_f \simeq 0.3$) on silica gel with chloroform-methanol, 10:1, vol/vol irrigant. Pigments **1**, **2** and **20** show intermediate behavior: more polar than the completely intramolecularly hydrogen bonded pigment, mesobilirubin-XIIIα and less polar than pigments that either cannot (etiobilirubin-IVγ) or do not (mesobilirubin-IVα) participate in intramolecular hydrogen bonding. Although we found **1** and **2** to be more soluble in chloroform than in methanol, **20** was more in methanol and less soluble in chloroform. Pigments **1**, **2** and **20** are insoluble in 5% aqueous sodium bicarbonate and sodium carbonate, like mesobilirubin-XIIIα and unlike mesobilirubin-IVα, which is soluble in both.

A more direct way to detect intramolecular hydrogen bonding is by analysis of the ^{1}H-nmr chemical shifts of pyrrole and lactam N-*H* signals, particularly in deuteriochloroform and dimethyl sulfoxide-d_6 solvents [11]. Dimethyl sulfoxide-d_6 exerts a levelling effect on the N-*H* resonances by participating in hydrogen bonding. Thus, as noted in Table 1, in this solvent the lactam N-*H* resonances are ~9.7-10 δ, and the pyrrole N-*H* resonances are 10.2-10.5 δ. One cannot make real distinctions between (non-vinyl) bilirubins that can participate in intramolecular hydrogen bonding, *e.g.*, mesobilirubin-XIIIα, and those that cannot because the propionic acids are absent, *e.g.*, etiobilirubin-IVγ, or because they are attached at the wrong place, *e.g.*, mesobilirubin-IVα. The N-*H* resonances of monopropionic acid analogs **1** and **20** provide no exception. Here, however, two sets of pyrrole N-*H* and lactam N-*H* resonances can be observed due to molecular dissymmetry.

In deuteriochloroform solvent, the N-*H* chemical shifts differ. We observed N-*H* to N-*D* exchange following addition of a drop of methanol-d_4, with the following relative rates of exchange for each peak: in **1** 10.53 ≥ 8.26 > 7.93 > 9.00; in **2** 9.31 > 10.23 > 9.92 > 8.35; in **20** 10.57 ≥ 8.00 > 8.85 > 7.90; in mesobilirubin-XIIIα, 10.59 > 9.15; and in etiobilirubin-IVγ, 10.58 > 10.28. Mesobilirubin-IVα was too insoluble in deuteriochloroform to carry out a similar measurement. The N-*H* signal assignments are made on the basis of the relative exchange rates, with lactam N-*H*'s exchanging faster than pyrrole N-*H*'s, and with hydrogen-bonded N-*H*'s exchanging slower than non-hydrogen bonded N-*H*'s [10,11].

Intramolecular hydrogen bonding, which is known [10,11] to be especially pronounced in deuteriochloroform for bilirubin-IXα and mesobilirubin-XIIIα (Figure 2) can also be detected by lactam N-*H* chemical shifts near 10.6 δ and pyrrole chemical shifts near 9.2 δ (Table 1) [11]. When *intra*molecular hydrogen bonding is not possible, as in etiobilirubin-IVγ, the pigment tends to engage in *inter*molecular hydrogen bonding in nonpolar/non-hydrogen bonding solvents, as has been shown for mesobilirubin-XIIIα dimethyl ester [11,17]. In this case the pyrrole N-*H* moves downfield by ~1 ppm. In **1**, **2** and **20**, one pyrrole and one lactam hydrogen move upfield by ~1-2 ppm, suggesting little if any hydrogen bonding to one dipyrrinone half of each molecule. However, one lactam N-*H* in each is deshielded to ~10.2-10.5 δ, consistent with residual hydrogen bonding. Although one lactam N-*H* resonance is approximately where it would be expected for both intramolecular and intermolecular hydrogen bonding, the second lactam in each is strongly shielded, which is not expected from intermolecular hydrogen bonding.

Table 2

Comparison of UV-Visible Spectral Data of Monopropionic Acid Bilirubin Analogs **1**, **2** and **20** with Mesobilirubin-XIIIα, Mesobilirubin-IVα and Etiobilirubin-IVγ

	λ max (ε)					
Solvent	**1**	**2**	**20**	Mesobilirubin-XIIIα	Mesobilirubin-IVα	Etiobilirubin-IVα
Benzene	419 (42,400) 397^{sh} (42,200)	421^{sh} (37,300) 390 (50,600)	415^{sh} (37,600) 396 (39,200)	435 (58,800) 417^{sh} (54,700)	419^{sh} (30,300) 390 (39,100)	417^{sh} (37,900) 396 (44,700)
Chloroform	414 (46,600)	417^{sh} (38,700) 392 (47,000)	414 (42,300)	431 (57,800)	418^{sh} (33,100) 390 (39,000)	421^{sh} (28,100) 383 (51,400)
Acetone	416 (43,100)	419^{sh} (38,700) 384 (47,400)	385 (40,400)	427 (56,200)	414^{sh} (30,400) 386 (36,900)	419^{sh} (35,200) 388 (42,700)
Acetonitrile	389 (41,700)	420^{sh} (25,800) 377 (55,000)	385 (40,200)	426 (56,500) 390^{sh} (46,300)	416^{sh} (21,900) 388 (43,300)	422^{sh} (20,800) 377 (60,000)
Methanol	427 (51,200) 399^{sh} (47,000)	423 (45,100) 397 (45,300)	426 (44,100) 392^{sh} (42,400)	426 (50,700) 401^{sh} (43,100)	420^{sh} (37,300) 394 (39,300)	430^{sh} (47,100) 398 (48,400)
Dimethyl Sulfoxide	426 (54,600) 396^{sh} (47,300)	424 (50,900) 396^{sh} (44,400)	428 (49,300) 395^{sh} (42,800)	426 (57,000) 397^{sh} (49,100)	426 (39,800) 396^{sh} (38,800)	426 (52,600) 397^{sh} (41,600)

^{sh} Shoulder.

The pyrrole N-*H* involved in intramolecular hydrogen bonding in **1** and **20** remains near 9 δ, as in mesobilirubin-XIIIα and bilirubin-IXα, and is somewhat more deshielded in **2**. In contrast, the pyrrole N-*H* not involved in hydrogen bonding is shifted markedly upfield. Intramolecular hydrogen bonding in **1** and **20** would be accommodated by a structure similar to that in Figure 2, but for **2** only a conformationally different structure would be necessary in order to accommodate intramolecular hydrogen bonding.

Further evidence on the conformation of **1**, **2** and **20** may be obtained from their uv-visible spectra (Table 2). Dimethylsulfoxide exerts a levelling effect such that for all pigments of Table 2, λ max lies near 426 nm, and the curve has a shoulder near 396 nm. Significant differences may be noted in other solvents. In methanol **1**, **20** and mesobilirubin-XIIIα exhibit similar λ max near 428 nm and a shoulder on the short wave length side of the band, but **2**, mesobilirubin-IVα and etiobilirubin-IVγ have the shoulder on the long wavelength side of the band and λ max near 396 nm. This distinction persists in acetonitrile, acetone and chloroform. Since mesobilirubin-IVα and etiobilirubin-IVγ are thought to be unable to participate in the type of conformation-determining *intra*molecular hydrogen bonding shown in Figure 1, they may adopt different conformations, perhaps dictated by *inter*molecular hydrogen bonding. Such differences in conformation are reflected in uv-visible spectra distinctively different from mesobilirubin-XIIIα, which is believed to participate in intramolecular hydrogen bonding as in Figure 1. And because the uv-visible spectra of **1** and **20** more closely resemble those of mesobilirubin-XIIIα, one is tempted to think that these three pigments adopt similar conformations. The conformation of **2**, however, is less clear. The nmr and solubility evidence suggests intramolecular hydrogen bonding, but the uv-visible evidence would point to ineffective intramolecular hydrogen bonding.

EXPERIMENTAL

General.

All nmr spectra were run on a Varian Unity Plus 500 MHz or GE QE-300 FT spectrometer in either deuteriochloroform (99.9% d_1) from Cambridge Isotope Labs, or dimethyl sulfoxide-d_6 (99.9% d_6), from Aldrich. Infrared spectra were run on a Perkin-Elmer 1600 FT-IR spectrophotometer. All uv-visible absorption spectra were run on a Perkin-Elmer model 3840 diode array instrument. Analytical thin layer chromatography (tlc) was carried out on J. T. Baker silica gel 1B-F plates (125 μ layer). Flash chromatography was accomplished on tlc grade silica gel (Woelm) with aspirator suction. Radial chromatography was carried out on preparative tlc grade Merck silica gel PF-254 with calcium sulfate binder using a Chromatotron (Harrison Research, Inc., Palo Alto, CA). High performance liquid chromatographic (hplc) analyses used a detector set at 420 nm for rubins and 390 nm for verdins and a Beckman-Altex Ultrasphere-IP 5 μm C-18 ODS column (25 x 0.46 cm), with a Beckman ODS precolumn (4.5 x 0.46 cm) and a flow of 1.00 ml/minute of 0.1 *M* di-*n*-octylamine acetate in 5% aqueous methanol as eluent [16]. Acetic acid, ethyl acetate, chloroform, dichloromethane and methanol were from Fisher. Disodium EDTA was from Aldrich. Aqueous formic acid (95%) was from J. T. Baker, and *p*-chloranil was from Eastman Kodak. Spectral grade acetone, acetonitrile, benzene, methanol, chloroform and dimethyl sulfoxide were from Fisher. All combustion microanalyses were performed by Desert Analytics, Tucson, AZ.

12-Despropionic Acid-12-ethyl-mesobiliverdin-XIIIα (**7**).

To a solution of methyl xanthobilirubinate (**12**) (549 mg, 1.74 mmoles) and kryptopyrromethenone (**13**) (427 mg, 1.73 mmoles) dissolved in hot dichloromethane (375 ml) was added *p*-chloranil (1.60 g, 6.51 mmoles) followed by 95% formic acid (30 ml). The reaction immediately turned green and was heated at reflux for 36 hours. The emerald green solution was concentrated to ~100 ml and worked up at -20° to precipitate out the reduced *p*-chloranil, which was removed by filtration and washed with cold dichloromethane. The combined washings were then cautiously washed with 5% aqueous sodium bicarbonate (3 x 100 ml), 1 *M* aqueous sodium hydroxide (3 x 100 ml), water (1 x 200 ml), saturated aqueous sodium chloride (1 x 200 ml) and dried over anhydrous sodium sulfate. The filtered solution was concentrated to dryness to afford a blue solid, which was dissolved in a minimum amount of chloroform and placed on a flash chromatography column (2.5 x 6.5 cm diameter) and eluted with chloroform:methanol (50:1) to remove any polar impurities. The blue eluent was concentrated to afford the mixed verdins as a blue solid (870 mg, 89%). The mixture was saponified without further purification.

To the mixed verdins (0.84 g) dissolved in hot methanol (500 ml) was added ascorbic acid (340 mg), disodium EDTA (~10 mg) and 2 *M* sodium hydroxide (175 ml). The solution was then heated to reflux with stirring under a blanket of nitrogen for 16 hours. The reaction was cooled, poured into acetic acid (100 ml) and *p*H 2.8 glycine hydrochloride buffer (600 ml) and extracted with chloroform until the aqueous layer was clear yellow. The blue organic extract was then washed with water (5 x 100 ml) and 1 *M* sodium hydroxide (until clear) to remove mesobiliverdin-XIIIα (**9**). (The latter could be recovered by acidifying with acid, dilute or concentrated hydrochloric acid). Further washing with water (2 x 100 ml), 1 M hydrochloric acid (2 x 100 ml), saturated aqueous sodium chloride and drying over anhydrous sodium sulfate to afford a blue solid after evaporation of solvent. The blue solid was dissolved in a minimum amount of chloroform, placed on a flash chromatography column (4 x 6.5 cm diameter) and eluted with chloroform:methanol (50:1) to remove etiobiliverdin-IVγ (**11**) (168 mg), then with chloroform:methanol (10:1) to remove the desired verdin **7** as a blue solid. It was purified further by radial chromatography, eluting with chloroform:methanol (50:1) to remove small amounts non-polar impurities, then eluting with chloroform:methanol (10:1) to remove **7** as a blue solid (132 mg). It had mp 138-140°; ir (film): ν 3210, 2969, 2932, 2863, 1963, 1586 cm^{-1}; uv-visible (chloroform): λ max 642 nm, ε, 14,300; λ max 370 nm, ε,

50,400; λ sh 305 nm, ε, 21,000; (methanol): λ max 641 nm, ε, 15,000; λ max 363 nm, ε, 50,900, λ sh 309 nm, ε, 21,800; (dimethyl sulfoxide): λ max 634 nm, ε, 16,300; λ max 372 nm, ε, 49,600; λ sh 316 nm, ε, 21,200; ^{1}H-nmr (deuteriochloroform): δ 1.09 (t, 3H, J = 7.5 Hz, CH_3), 1.24 (t, 6H, J = 7.5 and 7.7 Hz, CH_3), 1.84 (s, 3H, C-13 CH_3) 1.85 (s, 3H, C-7 CH_3), 1.99 (s, 3H, C-18 CH_3), 2.08 (s, 3H, C-2 CH_3), 2.12-2.66 (m, 10 H), 5.92 (s, 1H, C-15 CH), 6.06 (s, 1H, C-5 CH), 6.70 (s, 1H, C-10 CH), 9.0-9.5 (brs, 3H, NH), CO_2H not observed ppm; ^{13}C-nmr (deuteriochloroform): δ 8.25 (q), 8.32 (q), 9.30 (q), 9.61 (q), 14.52 (q), 14.55 (q), 16.06 (q), 17.74 (t), 17.84 (t), 17.91 (t), 19.99 (t), 35.17 (t), 96.49 (d), 97.23 (d), 114.5 (d), 126.9 (s), 128.1 (s), 128.3 (s), 128.4 (s), 138.9 (s), 139.2 (s), 139.2 (s), 140.3 (s), 141.6 (s), 141.7 (s), 146.7 (s), 147.2 (s), 147.3 (s), 151.7 (s), 172.6 (s), 174.5 (s), 176.7 (s) ppm.

Anal. Calcd. for $C_{32}H_{38}N_4O_4$ (542): C, 70.81; H, 7.06; N, 10.33. Found: C, 70.73; H, 6.98; N, 9.96.

12-Despropionic Acid-12-ethyl-mesobilirubin-XIIIα (**1**).

To a sonicating solution of **7** (36.4 mg, 0.067 mmole) dissolved in cold nitrogen-saturated methanol (15 ml) was added sodium borohydride (658 mg, 17.4 mmoles) in a single portion. The reaction turned yellow and sonication was allowed to continue for 1.5 hours. The reaction was then quenched by pouring into a solution of water (30 ml) and acetic acid (1 ml, 17.5 mmoles) and allowed to sonicate for 5 minutes. This yellow-green suspension was extracted with chloroform and the chloroform layer was washed with 0.1 *M* aqueous sodium bicarbonate (10 ml), water (10 ml) and dried over anhydrous sodium sulfate. After filtration and concentration to ~10 ml, the rubin solution was placed on a flash chromatography column (2.5 x 3.5 cm diameter). Elution with chloroform:methanol (10:1) removed a yellow band, which was collected and concentrated to afford a yellow-green solid. This was then triturated with cold methanol to remove any verdin impurities and the precipitate was collected by filtration as a yellow solid (24.9 mg, 0.046 mmole, 70%). It had mp 268-269° after blackening at 230-255°; ir (film): ν 3142, 2969, 2932, 2863, 1693, 1586 cm^{-1}; uv-visible data in Table 2; ^{1}H-nmr (deuteriochloroform): δ 1.10 (t, 9H, J = 7.5 Hz, CH_3), 1.87 (s, 6H, C-7 and C-13 CH_3), 2.10 (s, 3H, C-18 CH_3), 2.14 (s, 3H, C-2 CH_3), 2.51-2.82 (m, 10H), 3.99 (s, 2H, C-10 CH_2), 5.93 (s, 1H, C-15 CH), 6.10 (s, 1H, C-5 CH), 7.12 (brs, 1H, pyrrole NH), 7.65 (brs, 1H, lactam NH), 8.95 (brs, 1H, pyrrole NH), 10.49 (brs, 1H, lactam NH), 13.87 (brs, 1H, COOH) ppm; (dimethyl sulfoxide-d_6): δ 0.75 (t, 3H, J = 7.2 Hz, CH_3), 1.08 (t, 6H, J = 7.4 Hz, CH_3), 1.78 (s, 6H, C-7 and C-13 CH_3), 2.00 (s, 6H, C-2 and C-18 CH_3), 2.15-2.51 (m, 10H), 3.94 (s, 2H, C-10 CH_2), 5.95 (s, 1H, C-15 CH), 5.96 (s, 1H, C-5 CH), 9.78 (brs, 1H, lactam NH), 9.82 (brs, 1H, lactam NH), 10.29 (brs, 1H, pyrrole HN), 10.34 (brs, 1H, pyrrole NH), 11.92 (brs, 2H, COOH) ppm; ^{13}C-nmr (500 MHz) (dimethyl sulfoxide-d_6): δ 8.07 (2 x q), 9.11 (q), 9.15 (q), 14.82 (2 x q), 15.08 (q), 16.77 (q), 17.14 (t), 17.17 (t), 19.33 (t), 23.39 (t), 34.60 (t), 97.71 (d), 97.77 (d), 119.2 (s), 121.9 (s), 121.9 (s), 122.2 (s), 122.3 (s), 122.5 (s), 122.8 (s), 122.9 (s), 127.6 (s), 127.7 (s), 129.7 (s), 130.6 (s), 147.1 (s), 147.2 (s), 171.9 (s), 171.9 (s), 174.2 (s) ppm.

Anal. Calcd. for $C_{32}H_{40}N_4O_4$ (544): C, 70.56; H, 7.40; N, 10.29. Found: C, 70.16; H, 7.21; N, 9.89.

13-Despropionic Acid-13-methyl-12-desmethyl-12-ethyl-mesobiliverdin-IVα (**15**).

Methyl ψ-xanthobilirubinate (**14**) (549 mg, 1.74 mmoles) and kryptopyrromethenone (430 mg, 1.75 mmoles) were oxidatively coupled exactly as for **7** to afford **15** as a blue solid (126 mg). It had mp 207-209°; ir (film): ν 3143, 2969, 2934, 2873, 1700, 1648, 1589, 1220 cm^{-1}; uv-visible (chloroform): λ max 643 nm, ε, 13,000; λ sh 300 nm, ε, 18,200; λ max 368 nm, ε, 41,400; (methanol): λ max 643 nm, ε, 11,900; λ max 361 nm, ε, 41,600; (dimethyl sulfoxide): λ max 632 nm, ε, 12,500; λ max 370 nm, ε, 42,300; ^{1}H-nmr (500 MHz) (deuteriochloroform): δ 1.17 (t, 3H, J = 7.6 Hz), 1.23 (t, 3H, J = 7.6 Hz), 1.24 (t, 3H, J = 7.6 Hz), 1.818 (s, 3H), 1.824 (s, 3H), 2.07 (s, 3H), 2.08 (s, 3H), 2.08 (t, 2H, under CH_3 group), 2.47 (q, 2H, J = 7.6 Hz), 2.53 (q, 2H, J = 7.6 Hz), 2.59 (q, 2H, J = 7.6 Hz), 2.66 (t, 2H, J = 8.4 Hz), 5.82 (s, 1H), 6.00 (s, 1H), 6.64 (s, 1H), 9.34 (bs, 1H), 10.05 (bs, 2H) ppm; ^{13}C-nmr (500 MHz) (deuteriochloroform): δ 8.15 (q), 8.35 (q), 9.15 (q), 9.64 (q), 14.44 (q), 14.51 (q), 16.00 (q), 17.75 (t), 17.80 (t), 17.89 (t), 20.22 (t), 35.77 (t), 95.87 (d), 98.32 (d), 114.6 (d), 126.1 (s), 127.6 (s), 128.7 (2 x s), 132.8 (s), 136.6 (s), 137.6 (2 x s), 139.4 (s), 142.1 (s), 146.2 (s), 146.3 (s), 147.6 (s), 156.6 (s), 172.3 (s), 176.1 (s), 176.8 (s) ppm.

Anal. Calcd. for $C_{32}H_{38}N_4O_4$ (542): C, 70.81; H, 7.06; N, 10.33. Found: C, 70.48; H, 7.06; N, 10.08.

13-Despropionic Acid-13-methyl-12-desmethyl-12-ethyl-mesobilirubin-IVα (**2**).

To a sonicating solution of verdin **15** (50.7 mg, 0.093 mmole) dissolved in cold nitrogen-saturated methanol (20 ml) was added sodium borohydride (660 mg, 17.5 mmoles) in a single portion. The reaction turned yellow and sonication was allowed to continue for 20 minutes. The reaction was then quenched by pouring into water (20 ml) and acetic acid (1.00 ml, 17.5 mmoles) to give a yellow-green suspension. This was then extracted with dichloromethane, and the organic layer was washed with water (3 x 20 ml), concentrated to ~4 ml and purified by flash chromatography on silica gel (column 2.5 x 3.5 cm diameter) by eluting with chloroform:methanol (10:1) to remove a yellow band followed closely by some green bands. The yellow band was collected and concentrated to dryness to afford a yellow solid. This was then dissolved in hot methanol and cooled to 4° to give a green solid which was collected by filtration and washed with ice cold methanol to afford the rubin as a yellow-green solid (41.5 mg, 0.076 mmole, 82%). It had mp 167-169° dec; ir (film): ν 3355, 2964, 2919, 2861, 1648, 1625, 1267 cm^{-1}; uv-visible data in Table 2; ^{1}H-nmr (500 MHz) (deuteriochloroform): δ 1.09 (t, 6H, J = 7.4 Hz), 1.11 (t, 3H, J = 7.6 Hz), 1.64 (s, 3H), 1.77 (s, 3H), 2.04 (s, 3H), 2.09 (s, 3H), 2.45 (q, 4H, J = 7.4 Hz), 2.48 (q, 2H, J = 7.4 Hz), 2.62 (t, 2H, J = 6.7 Hz), 2.90 (t, 2H, J = 6.7 Hz), 4.07 (s, 2H), 6.02 (s, 1H), 6.09 (s, 1H), 8.35 (bs, 1H), 9.31 (bs, 1H), 9.92 (bs, 1H), 10.23 (bs, 1H), 12.20 (bs, 1H) ppm; ^{1}H-nmr (500 MHz) (dimethyl sulfoxide-d_6): δ 0.74 (t, 3H, 7.3 Hz), 1.08 (t, 3H, J = 7.1 Hz), 1.10 (t, 3H, 7.2 Hz), 1.75 (s, 3H), 1.78 (s, 6H), 1.78 (s, 6H), 2.00 (s, 3H), 2.16 (q, 2H, J = 7.3 Hz), 2.28 (t, 2H, J = 7.1 Hz), 2.50 (2 x q, 4H, under DMSO), 2.69 (t, 2H, J = 7.1 Hz), 3.91 (s, 2H), 5.96 (s, 1H), 6.06 (s, 1H), 9.80 (bs, 1H), 9.82 (bs, 1H), 10.33 (bs, 2H), 12.01 (bs, 1H) ppm; ^{13}C-nmr (500 MHz) (dimethyl sulfoxide-d_6): δ 8.05 (q), 8.07 (q), 8.49 (q), 9.09 (q), 14.72 (q), 14.84 (q), 15.01 (q), 16.74 (t), 17.18 (t), 17.22 (t), 19.53 (t), 23.60 (t), 35.43 (t), 97.80 (d), 97.86 (t), 115.1 (s), 121.7 (s), 121.8 (s), 122.3 (s), 122.4 (s), 122.8 (s), 122.9 (s), 126.6 (s), 127.6 (s), 127.7 (s), 129.7 (s), 130.3 (s), 147.2 (s), 147.4 (s),

171.95 (s), 171.96 (s), 173.9 (s) ppm.

Anal. Calcd. for $C_{32}H_{40}N_4O_4$ (544): C, 70.56; H, 7.40; N, 10.29. Found: C, 70.41; H, 7.28; H, 10.09.

12-Despropionic Acid-12-ethyl-α-methyl-mesobiliverdin-XIIIα.

To a solution of methyl (±)-α-methyl xanthobilirubinate (**19**) (552 mg, 1.74 mmoles) and kryptopyrromethenone (**13**) (430 mg, 1.75 mmoles) dissolved in hot dichloromethane (375 ml) was added *p*-chloranil (1.60 g, 6.5 mmoles) followed by 95 % formic acid (30 ml). The reaction immediately turned green and was heated at reflux for 48 hours. The reaction was worked up as in the preparation of **7** to afford a mixture of esters as a blue solid (905 mg, 92%). The mixture was saponified without further purification and worked up as in **7** to afford a blue solid.

The blue solid was then dissolved in a minimum amount of chloroform and placed on a flash column (4 x 6.5 cm diameter) and eluted with chloroform:methanol (50:1) to removed etiobiliverdin-IVγ (**11**), which was concentrated to dryness to afford a blue solid (202.4 mg). Further elution with chloroform-methanol (10:1) removed the desired verdin, which was concentrated to dryness to afford a blue solid (95.4 mg). It was found that when this flash chromatographic separation was repeated using chloroform-methanol-acetic acid (10:1:1, vol,vol,vol) traces of etiobiliverdin-IVγ and a yellow band (which previously ran at the tailing edge of the desired blue band) were removed. This improved separation made it possible to dispense with radial chromatography, and the yield of desired verdin was higher. The blue solid (95.4 mg, 13%) had mp 135-137°; ir (film): ν 3294, 2970, 2931, 2872, 1683, 1648, 1597 cm^{-1}; uv-visible (chloroform): λ max 641, ε, 13,700; λ sh 430 nm, ε, 11,700; λ max 372 nm, ε, 46,800; λ sh 312 nm, ε, 19,400; (methanol): λ max 633 nm, ε, 13,700; λ sh 426, ε, 12,700; λ max 366 nm, ε, 46,300; λ sh 306 nm, ε, 20,600; (dimethyl sulfoxide): λ max 633 nm, ε, 13,200; λ sh 428 nm, ε, 13,200; λ max 373 nm, ε, 48,100; ^{1}H-nmr (deuteriochloroform): δ 0.91 (d, 3H, J = 7.2 Hz), 1.06 (t, 3H, J = 7.4 Hz), 1.19 (t, 3H, J = 7.2 Hz), 1.20 (t, 3H, J = 7.2 Hz), 1.80 (s, 6H), 1.93 (s, 3H), 2.01 (s, 3H), 2.37-2.50 (m, 8H), 2.85 (m, 1H), 5.83 (s, 1H), 5.92 (s, 1H), 6.63 (s, 1H), 9.71 (bs, 1H), 11.15 (bs, 2H) ppm; ^{13}C-nmr (deuteriochloroform): δ 8.16 (q), 9.25 (q), 9.61 (q), 14.37 (2 x q), 15.94 (q), 17.75 (2 x q), 22.90 (t), 23.70 (t), 28.33 (t), 28.86 (t), 41.10 (d), 96.80 (d), 97.11 (d), 114.7 (d), 127.3 (s), 128.0 (s), 128.2 (s), 128.7 (s), 130.8 (s), 137.0 (s), 139.7 (s), 139.8 (s), 140.6 (s), 143.4 (s), 146.6 (s), 146.8 (s), 148.6 (s), 156.6 (s), 173.5 (s), 173.8 (s), 179.8 (s) ppm.

Anal. Calcd. for $C_{33}H_{40}N_4O_4$ (556): C, 71.18; H, 7.25; N, 10.07. Found: C, 71.12; H, 7.25; N, 9.70.

12-Despropionic Acid-12-ethyl-α-methyl-mesobilirubin-XIIIα (**20**).

A sonicating solution of the verdin above (53.0 mg, 0.098 mmole) dissolved in cold nitrogen-saturated methanol (15 ml) was reduced with sodium borohydride (659 mg, 17.4 mmoles) added in a single portion. The reaction turned yellow and sonication was allowed to continue for 20 minutes. The reaction was then quenched by pouring into a solution of water (30 ml) and acetic acid (1 ml, 17.5 mmoles) and allowed to cool to 4°. The resulting yellow-green precipitate was collected by centrifugation to afford a light yellow-green solid. This was then taken up in chloroform (30 ml), washed with water (3 x 20 ml), concentrated to ~4 ml and deposited on a flash chromatography column (2.5 x 3.5 cm diameter). Elution with chloroform:methanol (50:1) removed a yellow band, followed closely by some green bands. The yellow band was collected and afforded a yellow solid, which was dissolved in hot methanol (~23 ml). Addition of water dropwise precipitated the rubin. After cooling, the solid was collected by filtration and washed with water:methanol (10%) to afford a yellow-green solid rubin (30.5 mg, 0.056 mmole, yield 57%). It had mp 209-210°; ir (film): ν 3347, 3184, 2967, 2923, 2863, 1704, 1660, 1633 cm^{-1}; uv-visible spectral data in Table 2; ^{1}H-nmr (deuteriochloroform): δ 1.13 (t, 9H, J = 7.4 Hz, CH_3), 1.45 (d, 3H, J = 6.8 Hz), 1.87 (s, 6H), 2.10 (s, 3H), 2.12 (s, 3H), 2.43-2.89 (m, 9H), 4.15 and 3.83 (d of d, 2H, J = 16 Hz, AB-quartet), 5.94 (s, 1H), 6.10 (s, 1H), 7.77 (bs, 2H), 8.85 (bs, 1H), 10.54 (bs, 1H), 13.60 (bs, 1H) ppm; ^{1}H-nmr (500 MHz) (dimethyl sulfoxide-d_6): δ 0.74 (t, 3H, J = 7.3 Hz), 0.89 (d, 3H, J = 6.9 Hz), 1.08 (t, 6H, J = 7.6 Hz), 1.77 (s, 6H), 2.00 (s, 6H), 2.14-2.59 (m, 9H), 3.90 and 3.96 (d of d, 2H, J = 16.4 Hz, AB-quartet), 5.95 (s, 2H), 9.82 (bs, 2H), 10.28 (bs, 1H), 10.32 (bs, 1H), 11.39 (bs, 1H) ppm; ^{13}C-nmr (500 MHz) (dimethyl sulfoxide-d_6): δ 8.06 (2 x q), 9.11 (q), 9.47 (q), 14.82 (2 x q), 14.96 (q), 16.68 (2 x t), 16.75 (q), 17.15 (2 x t), 23.64 (t), 27.74 (d), 97.75 (2 x d), 118.30 (s), 121.9 (2 x s), 122.3 (s), 122.4 (s), 122.8 (s), 122.9 (s), 122.9 (s), 127.6 (s), 127.7 (s), 129.6 (s), 131.0 (s), 147.2 (s), 147.2 (s), 171.9 (s), 171.9 (s), 177.4 (s) ppm.

Anal. Calcd. for $C_{33}H_{42}N_4O_4$ (558): C, 70.93; H, 7.58; N, 10.03. Found: C, 70.83; H, 7.43; N, 10.17.

12-Despropionic Acid-12-ethyl-mesobilirubin-XIIIα Methyl Ester (**5**).

To a stirred suspension of **1** (7.70 mg, 0.014 mmole) in chloroform (20 ml) was added ethereal diazomethane (1 ml, ~0.42 *M*) and the resulting mixture was allowed to stir at room temperature for 10 minutes. Additional diazomethane (1 ml, ~0.42 *M*) was then added and stirring was continued for an additional 10 minutes. The solvent was evaporated by a stream of nitrogen gently blowing over the surface to afford a green solid which was further dried *in vacuo* (7.81 g, 0.014 mmoles, 100%) and had mp 249-252°; ir (film): ν 3346, 2964, 2923, 2864, 1737, 1660, 1632, 1462, 1368, 1172 cm^{-1}; uv-visible (chloroform): λ max 390 nm, ε, 32,200; λ sh 423 nm, ε, 21,700; (methanol): λ max 427 nm, ε, 31,800; λ sh 398 nm, ε, 30,500; (dimethyl sulfoxide): λ max 427 nm, ε, 36,200; λ sh 395 nm, ε, 30,800; ^{1}H-nmr (deuteriochloroform): δ 1.01 (t, 6H, J = 7.4 Hz, CH_3), 1.13 (t, 3H, J = 7.3 Hz, CH_3), 1.49 (s, 3H, C-13 CH_3), 1.50 (s, 3H, C-7 CH_3), 2.10 (s, 6H, C-2 and C-18 CH_3), 2.31-3.62 (m, 10H), 3.69 (s, 3H, $COOCH_3$), 4.13 (s, 2H, C-10 CH_2), 5.91 (s, 1H, C-15 CH), 5.94 (s, 1H, C-5 CH), 10.59 (brs, 2H, pyrrole NH), 10.24 (brs, 1H, lactam NH), 10.37 (brs, 1H, lactam NH) ppm; ^{13}C-nmr (deuteriochloroform): δ 7.67 (q), 7.70 (q), 9.45 (q), 9.50 (q), 14.56 (q), 14.59 (2 x q), 15.70 (t), 17.51 (t), 17.61 (t), 19.92 (t), 22.36 (t), 35.37 (t), 51.33 (q), 99.81 (d), 100.4 (d), 118.3 (s), 122.3 (s), 122.7 (s), 123.1 (s), 123.4 (s), 123.5 (s), 123.6 (s), 123.7 (s), 128.4 (s), 128.8 (s), 130.2 (s), 130.9 (s), 146.7 (s), 146.8 (s), 173.4 (s), 173.6 (s), 174.1 (s) ppm.

13-Despropionic Acid-13-methyl-12-desmethyl-12-ethyl-mesobilirubin-IVα Methyl Ester (**6**).

A stirred suspension of **2** (18.1 mg, 0.033 mmole) in chloroform (8 ml) was treated with etheral diazomethane and worked up exactly as described for **5**. The solvent was evaporated by a stream of nitrogen gently blowing over the sur-

face to afford a yellow-green solid. This was then taken up in a minimum amount of chloroform and flash chromatographed on silica, eluting with chloroforrn-methanol (50:1) to remove **6** compound as a yellow band, which was collected and concentrated to dryness. It had mp 193-195°; ir (film): ν 3353, 2966, 2933, 2871, 1735, 1661, 1634, 1458, 1368, 1284, 1246, 1172, 1060, 978, 757 cm^{-1}; uv-visible (chloroform): λ max 390 nm, ε, 41,400; λ sh 421 nm, ε, 25,100; (methanol): λ 396 nm, ε, 41,300; λ 421 nm, ε, 39,800; (dimethyl sulfoxide-d_6): λ 397 nm, ε, 39,500; λ 427 nm, ε, 42,500; ^{1}H-nmr (500 MHz) (deuteriochloroform): δ 1.03 (t, 6H, J = 7.4 Hz), 1.09 (t, 3H, J = 7.3 Hz), 1.54 (s, 3H), 1.56 (s, 3H), 2.10 (s, 3H), 2.11 (s, 3H), 2.37 (q, 4H, J = 7.4 Hz), 2.49 (t, 2H, J =7.6 Hz), 2.55 (q, 2H, J = 7.3 Hz), 2.84 (t, 2H, J = 7.6 Hz), 3.64 (s, 3H), 4.11 (s, 2H), 5.96 (s, 1H), 5.97 (s, 1H), 10.30 (s, 1H), 10.23 (s, 1H), 10.46 (bs, 2H) ppm; ^{13}C-nmr (500 MHz) (deuteriochloroform): δ 8.11 (q), 8.15 (q), 9.13 (q), 9.57 (q), 14.71 (q), 14.78 (q), 15.53 (q), 15.64 (q), 17.73 (t), 17.82 (t), 20.35 (t), 22.63 (t), 35.51 (t), 51.53 (q), 99.77 (d), 100.5 (d), 114.6 (s), 122.4 (s), 122.7 (s), 123.3 (s), 123.4 (s), 123.6 (s), 123.6 (s), 125.8 (s), 128.7 (s), 129.2 (s), 130.6 (s), 131.3 (s), 147.1 (s), 147.2 (s), 173.8 (s), 174.2 (2 x s) ppm.

12-Despropionic Acid-12-ethyl-α-methylmesobilirubin-XIIIα Methyl Ester.

To a stirred solution of **20** (8.50 mg, 0.016 mmole) in chloroform (8 ml) was treated with ethereal diazomethane and worked up exactly as described for the preparation of **5**. The product was dried *in vacuo* (8.92 mg, 0.016 mmole, 100%). It had mp 222-224°; ir (film): ν 3345, 2966, 2917, 1734, 1660, 1633, 1458, 1171 cm^{-1}; uv-visible (chloroform): λ max 388 nm, ε, 42,100; λ sh 419 nm, ε, 28,600; (methanol): λ max 425 nm, ε, 39,600; λ sh 395 nm, ε, 39,600; (dimethyl sulfoxide): λ max 427 nm, ε, 44,400; λ sh 397 nm, ε, 39,300; ^{1}H-nmr (deuteriochloroform): δ 1.01 (t, 6H, J = 7.5 Hz, CH_3), 1.11 (t, 3H, J = 6.0 Hz, CH_3), 1.25 (s, 3H, C-18 CH_3), 1.47 (s, 3H, C-2 CH_3), 2.09 (s, 3H, C-13 CH_3), 2.10 (s, 3H, C-8 CH_3), 2.13-3.57 (m, 10H), 3.67 (s, 3H, CO_2CH_3), 4.13 (s, 2H, C-10 CH_2), 5.92 (s, 1H, C-15 CH), 5.94 (s, 1H, C-5 CH), 10.19 (brs, 1H, pyrrole NH), 10.38 (brs, 1H, pyrrole NH), 10.56 (brs, 1H, lactam NH), 10.69 (brs, 1H, lactam NH) ppm; ^{13}C-nmr (deuteriochloroform): δ 7.79 (2 x q), 9.62 (q), 9.92 (q), 14.73 (q), 15.83 (2 x q), 16.41 (t), 17.76 (t), 22.68 (t), 28.51 (t), 29.68 (t), 40.88 (t), 51.53 (q), 100.1 (d), 100.4 (d), 117.5 (s), 122.6 (s), 122.9 (s), 123.2 (s), 123.6 (s), 123.7 (s), 123.8 (2 x s), 128.5 (s), 128.9 (s), 130.4 (s), 131.6 (s), 146.9 (s), 147.0 (s), 174.1 (s), 174.2 (s), 177.1 (s) ppm.

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