A SYNTHETIC BILIRUBIN IN A PORPHYRIN-LIKE CONFORMATION

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Abstract - An analog of bilirubin is held in a porphyrin-like shape by an N_{21},N_{24}-methano-bridge. In this conformation, the two propionic acid groups cannot participate in intramolecular hydrogen bonding, and the pigment is thus much more polar and hydrophilic than bilirubin. The uv-visible spectra (ϵ^{max} 20,400, λ^{max} 381 nm in benzene and ϵ^{max} 23,800, λ^{max} 385 nm) are nearly invariant over the solvent polarity range. Circular dichroism of the pigment complex with human serum albumin gives a weak bisignate Cotton effect: $\Delta\epsilon^{max}_{422}$ = -4.7, $\Delta\epsilon^{max}_{366}$ = +3.7 in pH 7.4 buffer.

Complicated, structurally interesting linear tetrapyrroles such as the natural product bilirubin (Figure 1A) are formed in animal metabolism from normal turnover of hemoglobin and other heme proteins.[1-3] Considerable effort has been devoted to understanding the properties and metabolism of bilirubin, the yellow, neurotoxic pigment of jaundice, with particular attention being focussed on its unique ability to fold into a conformation where the carboxylic acid groups are sequestered through intramolecular hydrogen bonding (Figure 1D).[4-6] Hydrogen bonding that lowers their acidity, decreases the polarity of the pigment and renders it unexcretable in normal metabolism, except by glucuronidation. Bilirubin analogs, *e.g.*, mesobilirubin-IVα (Figure 1B), with propionic acid groups relocated from their natural sites at C_8 and C_{12} are more polar than bilirubin and do not require glucuronidation for hepatic excretion.[7,8] However, bilirubin analogs with propionic acid groups at C_8 and C_{12}, *e.g.*, mesobilirubin-XIIIα (Figure 1C), typically exhibit the same unique polarity and excretability properties as bilirubin because they can tuck their carboxylic acid groups inward, where they are tethered to an opposing dipyrrinone by intramolecular hydrogen bonding.

Such intramolecular hydrogen bonding is central to our understanding of bilirubin stereochemistry, solution properties and metabolism.[1,4-7,9] Although the component dipyrrinones may rotate relatively independently about the central -CH_2- group, to produce a large number of conformations, only a few have the unique ridge-tile shape required for intramolecular hydrogen bonding: the structure shown in Figure 1D and its non-superimposable mirror image. These two enantiomeric conformations, although stabilized by a network of intramolecular hydrogen bonds, are in dynamic equilibrium, interconverting fairly rapidly at room temperature over a barrier of ~20 kcal/mole.[5,6] Our interest in defining pigment stereochemistry through mechanisms other than by intramolecular hydrogen bonding between propionic acid and dipyrrinone groups led us to consider tethering the lactam nitrogens (N_{21} and N_{24}) to a common alkyl chain. This can be expected to hold the tetrapyrrole in a helical porphyrin-like shape, with chains of differing lengths giving helices with differing pitch. Intramolecular hydrogen bonding should become impossible, and the helical bilirubin analog can thus be expected to exhibit very different solution

Dedicated to Professor Alan R. Katritzky on the occasion of his 65th birthday.

and spectrosopic properties. In the following, we report on the synthesis, properties and conformational analysis of a new, symmetric bilirubin analog: N_{21},N_{24}-methanomesobilirubin-XIIIα (**1**) and its dimethyl ester (**2**).

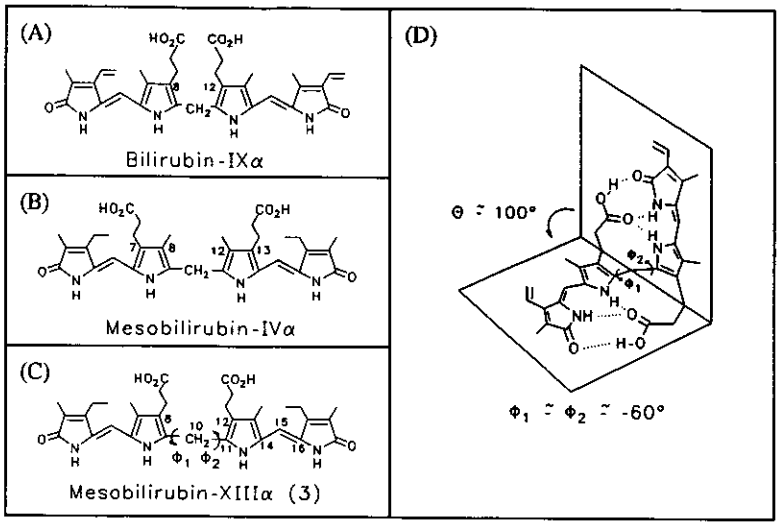
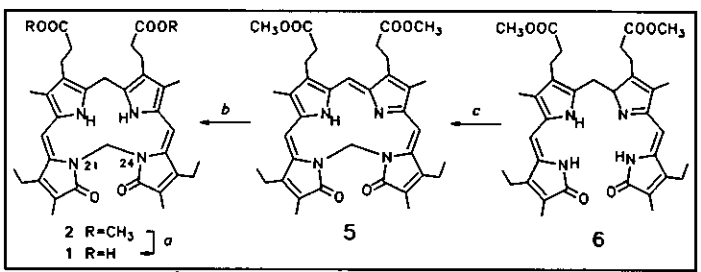


Figure 1. (A) Linear representation of bilirubin-IXα. (B) Mesobilirubin-IVα. (C) Mesobilirubin-XIIIα (**3**). (D) Folded, intramolecularly hydrogen bonded conformation, $\phi_1 \simeq \phi_2 \simeq -60°$, shaped like a ridge-tile and corresponding to a global energy minimum conformation. The interplanar dihedral angle $\theta \simeq 100°$. Only one of two enantiomeric conformations is shown.

RESULTS AND DISCUSSION

Synthesis. The target, N_{21},N_{24}-methano-mesobilirubin-XIIIα (**1**), was prepared in three steps from mesobiliverdin-XIIIα dimethyl ester (**6**)[10] as outlined (right). The method is similar to that employed by Falk and Thirring[11] to prepare N_{21},N_{24}-methano-etiobilirubin-IVγ, which has no carboxylic acid or ester groups. Thus, reaction of **6** in dimethylformamide with CH_2I_2 and sodium methoxide was accomplished to yield the desired N_{21},N_{24}-methano-bridged verdin (**5**) in 25% yield in addition to a minor amount ~1% of the addition to the N_{21},N_{22}-bridged product. The mixture was separated by flash chromatography on silica gel.



[a]NaOH/aq. THF; [b]$NaBH_4$/THF/CH_3OH; [c]CH_2I_2/$HCON(CH_3)_2$/$NaOCH_3$.

Polarity from Chromatographic Behavior. N_{21},N_{24}-Methanomesobilirubin-XIIIα (**1**) has a shorter retention time (~5.5 min) when coinjected with the parent mesobilirubin-XIIIα (**3**) (~17.3 min) on reverse phase hplc, suggesting that **1** is more polar than the parent. And on silica gel tlc, **1** has a much smaller R_f value (0.0) when compared with the mesobilirubin-XIIIα (**3**) standard (R_f=0.94) using CH_2Cl_2: CH_3OH (100:3, vol/vol) as eluant, confirming that **1** is much more polar than **3**.

Structure from NMR. The constitutional structure of the N_{21},N_{24}-methano-rubins (**1**) and (**2**) are consistent with their ^{13}C-nmr (Table 1). Thus, for N_{21},N_{24}-methano-mesobilirubin-XIIIα (**1**) and its dimethyl ester (**2**) the carbon

chemical shifts are essentially identical, and they are also similar to those of the parent mesobilirubin-XIIIα (**3**) and its dimethyl ester. They differ in one major way: The presence of a new carbon signal from the N_{21}-CH_2-N_{24} bridge is found in **1** and in **3**. Although the chemical shifts of the carbons in the β-substituents are nearly the same **1** and **3**, and in **2** and **4**, somewhat larger differences may be noted in comparing the carbon chemical shifts of the tetrapyrrole nucleus. Such differences probably reflect changes in shieldings due to the presence of the N_{21}-CH_2-N_{24} bridge, which restricts the shape of the pigment to a new conformation, one very different from that of the unbridged parents, **3** and **4**. For example, the meso carbons 5, 10 and 15 in **1** and **2** are shielded by ~1 ppm relative to those if **3** and **4**. Other skeletal carbons are comparably shifted, with greater shieldings coming from the lactam ring carbons forced to lie above or below the lactam C=O groups, *cf.* C_1/C_{19}.

Table 1. Comparison of the ^{13}C-nmr Chemical Shifts and Assignments for N_{21},N_{24}-Methano-mesobilirubin-XIIIα (**1**), Its Dimethyl Ester (**2**), Mesobilirubin-XIIIα (**3**) and Its Dimethyl Ester (**4**) in $(CD_3)_2SO$.[a]

Position	Carbon	δ for **1**	δ for **3**	δ for **2**	δ for **4**
1,19	C=O	169.39	172.42	169.39	172.37
2,18	=C-	118.63	122.42	118.48	122.30
2^1,18^1	CH_3	8.14	8.54	8.09	8.52
3,17	=C-	146.21	141.67	146.21	147.64
3^1,17^1	CH_2	17.44	17.64	17.39	17.61
3^2,17^2	CH_3	14.77	15.29	14.70	15.29
4,16	=C-	127.62	128.28	127.67	128.22
5,15	=CH-	99.13	98.19	99.04	98.09
6,14	=C-	121.41	119.72	121.81	123.04
7,13	=C-	119.67	123.00	119.30	119.36
7^1,13^1	CH_3	9.62	9.62	9.52	9.48
8,12	=C-	123.77	123.39	123.85	123.37
8^1,12^1	CH_2	19.78	19.74	19.61	19.68
8^2,12^2	CH_2	35.60	34.81	35.17	34.26
8^3,12^3	C=O	174.32	174.46	173.03	173.17
8^4,12^4	OCH_3	—	—	51.13	51.41
9,11	=C-	136.21	130.81	135.38	130.83
10	CH_2	22.28	23.96	22.03	23.99
N_{21},N_{24}	-CH_2-	54.82	—	54.64	—

[a]Run at 2.5 x10^{-2} M concentration of pigment at 22°C. Chemical shifts are in ppm downfield from $(CH_3)_4Si$.

NMR Analysis and Intramolecular Hydrogen Bonding. The ^{1}H-nmr N-*H* chemical shifts of the pyrrole and lactam have proven to be an excellent way to determine whether the dipyrrinone units of bilirubins are involved in intramolecular hydrogen bonding.[12,13] Previous studies have shown that the pyrrole N-*H* appears near 9.2 δ in $CDCl_3$ solvent (*e.g.* for **3**) when the dipyrrinone and carboxylic acid groups are intramolecularly hydrogen bonded, as shown in Figure 1.[12,13] When **3** is esterified, however, or when its propionic acid groups are relocated to C-7 and C-13 (as in mesobilirubin-IVα), or when they are replaced by ethyl (as in etiobilirubin-IVγ), the

pyrrole hydrogens become more deshielded (10.3 δ) due to dipyrrinone-dipyrrinone intermolecular hydrogen bonding (Table 2).[14,15] In $(CD_3)_2SO$, all of the dipyrrinone N-*H*'s become hydrogen bonded to solvent; so, the distinctions due to self-association and intramolecular hydrogen bonding seen in $CDCl_3$ are lost, and all pyrrole N-*H* resonances appear near 10.4 δ. It was anticipated that since the pyrrole N*H*'s of **1** and **2** cannot become involved in hydrogen bonding the chemical shifts of **1** and **2** would be strongly shielded. This is observed for $CDCl_3$ solutions, but in $(CD_3)_2SO$ solvent (Table 2) the chemical shifts are much less strongly shielded, suggesting that the sulfoxide hydrogen bonds to the pyrrole N-*H*'s even in a sterically crowded environment. Unlike **3** and **4**, which exhibit different N*H* chemical shifts in $CDCl_3$, **1** and **2** show essentially no difference. The picture is one where the conformations of **1** and **2** are held fixed in a helical porphyrin-like shape by the N_{21},N_{24}-methano bridge.

Table 2. Comparison of Bridged and Unbridged Mesobilirubin Lactam and Pyrrole N-*H* Chemical Shifts[a] in $CDCl_3$ and $(CD_3)_2SO$ Solvents.[b]

Pigment	$CDCl_3$			$(CD_3)_2SO$		
	Lactam	Pyrrole	CO_2H	Lactam	Pyrrole	CO_2H
N_{21},N_{24}-Methanomesobilirubin-XIIIα (**1**)	—	insol.	insol.	—	9.64	12.06
Mesobilirubin-XIIIα (**3**)	10.57	9.15	13.62	9.72	10.27	11.87
N_{21},N_{24}-Methanomesobilirubin-XIIIα Dimethyl Ester (**2**)	—	7.89	—	—	9.64	—
Mesobilirubin-XIIIα Dimethyl Ester (**4**)	10.54	10.27	—	9.74	10.40	—
N_{21},N_{24}-Methanoetiobilirubin-IVγ	—	7.39[c]	—	—	NA	—

[a]δ, ppm downfield from $(CH_3)_4Si$. [b]Run as 10^{-2} *M* $(CD_3)_2SO$ and 10^{-3} *M* $CDCl_3$ solutions at 22°C. [c]Ref. 11.

UV-Visible Spectral Analysis and Conformation from Exciton Coupling. Further evidence on conformation comes from solvent-dependent uv-visible spectra. Over a wide range of solvents with varying polarity and hydrogen bonding ability (benzene, chloroform, methanol and dimethylsulfoxide), the uv-visible spectra of mesobilirubin-XIIIα (**3**) change very little, with λ^{max} being near 430 nm and λ^{sh} near 395 nm[10,15] — corresponding to the two exciton components from electric transition dipole-dipole interaction of the two proximal dipyrrinone chromophores approximately 90° apart (as in Figure 1D).[16-18] Since **3** is known from nmr studies to adopt the intramolecularly hydrogen bonded conformation of Figure 1D in $CDCl_3$ solvent and a similar conformation in $(CD_3)_2SO$ solvent,[5,19] it might be argued that a uv-visible exciton couplet with $\lambda^{max} \simeq 430$ nm, $\lambda^{sh} \simeq 395$ nm can be taken as an indicator of a folded (but not necessarily hydrogen-bonded) conformation akin to that of Figure 1D. The uv-visible spectra of **1** and **2** (Table 3) contrast strongly with those of **3** and **4**. The observed λ^{max} of **1** is shifted to the blue by 40-50 nm relative to **3**, and ϵ^{max} is reduced by 50% or more. This blue shift is expected from exciton coupling theory[18] when the pigment adopts a conformation in which the dipyrrinone component chromophores are oriented with nearly parallel transition dipoles. The data for **1** are thus fully consistent with a porphyrin-like conformation; whereas, the data for **3** are consistent with a folded ridge-tile shape.

As might be expected, the uv-visible spectral of the N_{21},N_{24}-bridged dimethyl ester (**2**) is quite comparable to that of its parent acid (**1**). Very little solvent dependence is found (Table 3) in contrast to **4**, which typically exhibits a strong solvent, concentration and temperature dependence due to formation of dimers[20] in non-polar solvents, such as benzene and chloroform, that exhibit a narrow bandwidth intense absorption at λ^{max} near 380 nm and weak shoulder at λ^{sh} near 430 nm.[10,15] In more polar solvents such as CH_3OH and $(CH_3)_2SO$ the solutions are

largely monomeric, and the uv-visible spectra are thus quite similar to those of the parent acid in these solvents, with λ^{max} near 435 nm and λ^{sh} near 400 nm.[10,13a] Unlike **4**, **2** shows none of these characteristic shifts but exhibits the same solvent independent broad uv-visible absorption as the parent acid (**1**) with λ^{max} near 380 nm. The uv-visible spectra of **1** and **2** provide corroborating evidence for porphyrin-like structures, as the uv-visible λ^{max} are blue shifted, as is expected for an exciton system where the component chromophores are positioned such that the relevant electric transition moments approach a parallel alignment. In the folded conformation (Figure 1D) the transition moments are not parallel, and the uv-visible maximum is red-shifted. Since the uv-visible spectra of **2** and **4** are similar, and the structure of **2** is known to be confined to the porphyrin-like conformation, it seems likely that mesobilirubin (and bilirubin) dimethyl esters also adopt a porphyrin-like conformation.

Table 3. Comparison of UV-Visible Spectral Data [a] for N_{21},N_{24}-Methanomesobilirubin-XIIIα (**1**) and Its Dimethyl Ester (**2**) With Mesobilirubin-XIIIα (**3**) and Its Dimthyl Ester (**4**).

Solvent	UV-Visible λ^{max} (ϵ^{max})			
	1	**2**	**3**	**4**
C_6H_6	381 (20,400)	381 (21,000)	435 (52,100)	380 (60,700)
CH_2Cl_2	396 (22,800)	382 (21,400)	431 (53,200)	376 (62,600)
$CHCl_3$	390 (19,800)	379 (20,900)	431 (52,800)	382 (59,300)
$(CH_3)_2CO$	378 (20,100)	378 (23,700)	428 (50,400)	377 (51,500)
CH_3CN	377 (20,700)	379 (22,100)	425 (49,300)	374 (60,400) 422^{sh} (15,600)
CH_3OH	384 (23,100)	383 (26,000)	426 (51,600) 401^{sh} (43,300)	428 (57,500) 396^{sh} (42,400)
$(CH_3)_2SO$	385 (23,800)	385 (26,500)	426 (49,300) 397^{sh} (47,200)	430 (61,500) 396^{sh} (42,800)

[a]Run at 1-2 x10^{-5} *M* concentrations; λ^{max} and λ^{sh} in nm; ϵ^{max} and ϵ^{sh} in L · mole · cm^{-1}.

Induced Circular Dichroism and Binding to Albumin. A solution of **1** in buffered solution with 2 mole equivalents of human serum albumin (HSA) gives a bisignate circular dichroism (CD) spectrum for the long wavelength uv-visible transition. The CD is opposite in sign and much weaker in magnitude than that observed for mesobilirubin-XIIIα (Table 4). When bound to HSA and other species' serum albumin, bilirubin-IXα is known to exhibit optical activity, seen typically as an induced circular dichroism (CD), which is usually intense and bisignate.[21,22] The origin of the optical activity comes from the pigment adopting a chiral conformation selected at the binding site on the protein. The bisignate CD comes from exciton coupling of two electric dipole transitions: one from each of the pigment's twin dipyrrinone chromophores, *viz.* those from the long wavelength uv-visible excitation near 410 nm. The protein acts as an enantioselective binding agent and constrains the pigment to adopt a chiral conformation, and as reported previously,[21] the presence of at least one propionic acid group at C_8 or C_{12} is essential to the enantioselectivity in binding. Thus, bilirubin pigments with both propionic acid groups esterified as methyl esters give only very weak induced CDs. As expected, therefore, diester (**2**) gave only a negligible CD (Table 4), as does mesobilirubin-XIIIα dimethyl ester. In contrast, **1** gives a far stronger bisignate CD, but weaker than that of **3**. This finding reinforces the notion that CO_2H groups are essential to the enantioselectivity by the protein, even when they are not involved in intramolecular hydrogen bonding. Whether

the maximum possible CD magnitudes in **1** are inherently much less than those from **3** is not known. And whether the albumin is less enantioselective in its binding of **1** as compared with **3** is as yet unclear. In favor of the former explanation, in an organic solvent ($CHCl_3$) with an optically active amine, (-)-ephedrine, as the chiral complexing agent, the very large induced CD Cotton effects found for **3** are not marked by those of **1** (Table 4), which remain comparable to the Cotton effects induced by HSA.

Table 4. Comparison of Circular Dichroism and UV-visible Spectral Data[a] for N_{21}-N_{24}Methanomesobilirubin-XIIIα (**1**), Its Dimethyl Ester (**2**), and Mesobilirubin-XIIIα (**3**) in (A) pH 7.4 Buffered Aqueous Human Serum Albumin (HSA) Solutions Containing 1% Dimethylsulfoxide,[b] and (B) Chloroform Plus (1*R*,2*S*)-(-)-Ephedrine.

Pigment	Circular Dichroism			UV-Visible
	$\Delta\epsilon^{max}$ (λ_1)	λ at $\Delta\epsilon=0$	$\Delta\epsilon^{max}$ (λ_2)	ϵ^{max} (λ)
N_{21}-N_{24} Methano-mesobilirubin-XIIIα (**1**)	(A) -4.7 (422)[c] (B) -15.1 (423)	386 383	+3.7 (366) +4.8 (360)	16,000 (378) 16,300 (378)
Mesobilirubin-XIIIα (**3**)	(A) +35 (444) (B) -110 (447)	408 419	-35 (392) +87 (402)	43,000 (435) 40,000 (438)[sh]
N_{21}-N_{24}-Methanomesobilirubin-XIIIα Dimethyl Ester (**2**)	(A) <1.0 (411) (B) <0.1 (411)	382 —	<0.5 (368) <0.1 (368)	19,000 (379) 18,900 (379)

[a]$\Delta\epsilon$ and ϵ in L · $mole^{-1}$ · cm^{-1} and λ in nm. [b]For 2-3 x10^{-5} *M* pigment solutions run with 2 mole equivalents of HSA, 1%$(CH_3)_2SO$ has no effect on the BR-HSA CD spectrum. [c]1000:1 molar ratio amine:pigment.

CONCLUDING COMMENTS

Intramolecular hydrogen bonding between propionic acid CO_2H and dipyrrinone groups is known to be a dominant, conformation stabilizing force in bilirubin and its analogs.[17] The current study shows that new and interesting, much more polar bilirubin analogs can be prepared by linking the lactam nitrogens with a -CH_2- group. The resulting N_{21},N_{24}-methano-bridged pigment adopts either of two doughnut-shape enantiomeric conformations in which the dipyrrinones are highly twisted. In the presence of molecular recognition agents, such as HSA or (-)-ephedrine, the methano bridged acid (but its dimethyl (ester) exhibits a moderate induced bisignate CD.

EXPERIMENTAL

General Procedures. All ultraviolet-visible spectra were recorded on a Perkin Elmer Model 3840 diode array or Cary 219 spectrophotometer, and all circular dichroism (CD) spectra were recorded on a JASCO J-600 instrument. Nuclear magnetic resonance (nmr) spectra were determined on a GE QE-300 300-MHz spectrometer in $CDCl_3$ solvent (unless otherwise specified) and reported in δ ppm downfield from $(CH_3)_4Si$. Melting points were determined on a Mel-Temp capillary apparatus and are uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ. High resolution mass spectra were run at the Midwest Center for Mass Spectrometry, University of Nebraska, Lincoln. Analytical thin layer chromatography was carried out on J.T. Baker silica gel IB-F plates (125 μ layer). 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 $CaSO_4$ preparative thin layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA). Hplc analyses were carried out on a Perkin-Elmer Series 4 high performance liquid chromatograph with an LC-95 uv-visible spectrophotometric detector (set at 410 nm) equipped with a Beckman-Altex ultrasphere-IP 5 μm C-18 ODS column (25 x 0.46

cm) and a Beckman ODS precolumn (4.5 x 0.46 cm). The flow rate was 1.0 mL/minute, and the elution solvent was 0.1 *M* di-*n*-octylamine acetate in 5% aqueous methanol (pH 7.7, 31°C). Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). Diiodomethane, tetrahydrofuran, acetonitrile, dimethyl sulfoxide, sodium methoxide and sodium borohydride, were from Aldrich. Tetrahydrofuran was dried by distillation from sodium; methanol was dried (Mg, reflux) and distilled. Solutions of N_{21},N_{24}-methanomesobilirubin-XIIIα (**1**), its dimethyl ester (**2**), mesobilirubin-XIIIα (**3**) and its dimethyl ester (**4**) in pH 7.4 aqueous human serum albumin were prepared as reported earlier,[21] except the weighed pigment and albumin were mixed together in 0.3 ml of dimethyl sulfoxide, cooled in ice, then diluted with Tris buffer to a final volume of 10 ml.

N_{21},N_{24}-Methanomesobiliverdin-XIIIα Dimethyl Ester (5).[10] Mesobiliverdin-XIIIα dimethyl ester (123 mg, 0.2 mmol) was dissolved in 10 ml of absolute N,N-dimethylformamide (N_2-saturated) and heated to 100°C under N_2 in the dark. To the mixture, sodium methoxide (82 mg, 1.52 mmol) was added and stirred for 10 min. Diiodomethane (0.7 ml) was then added to the mixture dropwise *via* syringe, and the mixture was stirred for an additional 10 min. The hot reaction mixtures was poured into a solution of 100 ml of water/ice and chloroform (20 ml). The organic phase was collected and aqueous layer was washed several times with chloroform (20 ml each) until colorless. The combined organic layers were washed with cold water (5 x100 ml) and saturated NaCl solution (1 x100 ml). The washed organic layer was dried over Na_2SO_4, filtered, and the solvent was removed (rotary evaporator). The residue was flash chromatographed on silica gel ($CHCl_3$-CH_3OH, 100:3 v/v) to give 29 mg (24%) of pure green product. It had mp 220°C (decomp.); uv-vis, ϵ_{359}^{max} 67,600 ($CHCl_3$), ϵ_{699}^{max} 11,600 ($CHCl_3$); ir ($CHCl_3$), ν: 2974, 2878, 1733, 1693 cm^{-1}; ^{1}H-nmr δ: 1.163 (4, 6H, J=7.5 Hz), 1.758 (s, 6H), 2.074 (s, 6H), 2.519 (m, 8H, J=7.2 Hz), 3.076 (s, 6H), 5.937 (s, 2H), 6.678 (s, 1H), 6.928 (s, 2H), 13.479 (s, 1H) ppm; ^{13}C-nmr δ: 9.39 (q), 9.99 (q), 14.75 (q), 18.93 (t), 20.52 (t), 35.93 (t), 52.35 (q), 58.71 (t), 100.85 (d), 116.20 (d), 126.89 (s), 129.01 (s), 136.91 (s), 142.07 (s), 146.15 (s), 148.31 (s), 151.74 (s), 173.73 (s), 177.43 (s) ppm. *Anal.* Calcd for $C_{35}H_{42}N_4O_6 \cdot$ ¼ H_2O: C, 68.11; H, 6.67; N, 8.83. Found: C, 67.73; H, 6.42; N, 8.85.

N_{21},N_{24}-Methanomesobilirubin-XIIIα Dimethyl Ester (2). N_{21},N_{24}-Methanomesobiliverdin dimethyl ester (100 mg, 0.16 mmol) was dissolved in nitrogen-saturated tetrahydrofuran (50 ml) at 0°C. After the addition of sodium borohydride (0.6 g, 16 mmol) in one portion, methenol (13 ml) was added dropwise to the mixture at 0°C in the dark under a nitrogen atmosphere. The resulted yellow solution was stirred an additional 30 min. at 0°C. The mixture was then neutralized carefully with 10% aqueous HCl at 0°C. After the addition of water (50 ml), the mixture was extracted with dichloromethane until the aqueous layer became almost colorless. The combined organic layers were washed with water (2x100 ml), saturated aqueous NaCl solution (1x50 ml), dried over $MgSO_4$ and filtered. After the evaporation of the solvent (rotary evaporator), the yellow product was chromatographed (CH_2Cl_2-MeOH, 100:3, v/v) to give the 87 mg of desired product (85% yield). It had mp >90°C (decomp.); ir ($CHCl_3$), ν: 3438, 2953, 2877, 1726, 1687 cm^{-1}; ^{1}H-nmr δ: 1.130 (t, 6H, J=7.5 Hz), 1.771 (s, 6H), 2.019 (s, 6H), 2.488 (m, 4H), 2.576 (m, 4H), 2.803 (m, 4H), 3.509 (s, 6H), 3.900 (s, 2H), 4.654 (s, 2H), 6.061 (s, 2H), 7.894 (s, 2H) ppm; ^{13}C-nmr data are in Table 1; mass spectra, *m/z* (rel. intens.): 628 (23), 493 (9), 328 (19), 327 (31), 326 (33), 316 (12), 315 (48), 314 (100), 241 (24) amu. *HRms Anal.* Calcd for $C_{36}H_{44}N_4O_6$: 628.32606. Found: 628.32912.

N_{21},N_{24}-Methanomesobilirubin-XIIIα (1). N_{21},N_{24}-Methanomesobilirubin dimethyl ester (50 mg, 0.8 mmol) was heated to 50°C in 13 ml of tetrahydrofuran in the presence of 1 *M* aqueous NaOH solution (0.7 ml) under a nitrogen atmosphere. After cooling to room temperature, the solvent was evaporated (rotary evaporator), and the residue was dissolved in degassed water. The aqueous layer was washed with $CHCl_3$ until the organic layer

became colorless, to remove unreacted ester. The aqueous layer was then neutralized with 10% HCl carefully at 0°C. The resultant solid was collected by centrifugation, washed with cold, degassed water several times and dried under vacuum to give a yellow-brown solid: 39 mg, 82% yield. It had mp >150°C (decomp); ir (KBr) ν: 3478, 2968, 1742, 1694, 1652 cm^{-1}; ^{1}H-nmr ($(CD_3)_2SO$) δ: 1.065 (t, 6H, J=7.5 Hz), 1.657 (s, 6H), 2.013 (s, 6H), 2.373 (m, 4H), 2.400 (mixed with solvent peaks), 2.620 (m, 4H), 3.771 (s, 2H), 4.892 (s, 2H), 6.115 (s, 2H), 9.643 (s, 2H), 12.058 (s, 2H) ppm; ^{13}C-nmr data are in Table 1; *FAB HRms Anal.* Calcd for $C_{34}H_{40}N_4O_6Na$: 623.2836. Found: 623.2814.

ACKNOWLEDGEMENTS.
We thank the National Institutes of Health (HD17779) for generous support.

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Received, 10th September, 1993