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SYNTHESIS AND UNUSUAL PROPERTIES OF C(10)-*gem*-DIMETHYL BILIRUBIN ANALOGS

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Abstract. The characteristic thermodynamically-favored intramolecularly hydrogen-bonded conformation adopted by bilirubin pigments is destabilized by substituting methyl groups on the C(10) central methylene These methyl groups impose conformation-destabilizing methyl-methylene non-bonded steric interactions with the propionic acid β-CH_2 groups at C(8) and C(12) when the propionic acids are engaged in intramolecular hydrogen bonding with the opposing dipyrrinones Amphiphilic 10,10-dimethylbilirubins (1) and (2) are found to be more polar, but also more soluble than the parents (3) and (4) in organic solvents, yet, ^{1}H-NMR studies in non-polar solvents indicate that a deformed but folded, intramolecularly hydrogen-bonded conformation is retained The dimethyl esters of 10,10-dimethylbilirubins 1 and 2 did not exhibit the typical strong tendency of bilirubin dimethyl esters to form intermolecular hydrogen bonds in non-polar solvents such as chloroform and benzene

INTRODUCTION

In normal human metabolism ~300 mg/day/individual of bilirubin-IXα (Figure 1), the yellow-orange cytotoxic pigment of jaundice, is produced and excreted [1,2] Despite its implication in a diverse array of protein binding situations involved in its transport and metabolism,[3,4] the conformation of the pigment in solution, or when bound to proteins or in lipid matrices is incompletely understood However, it is becoming increasingly clear that bilirubin exhibits a special tendency to adopt a ridge-tile conformation which is further stabilized by intramolecular hydrogen bonding (Figure 1) [1,5-10] It is apparently in this pre-organized structure that bilirubin is able to cross several selective physiologic barriers the placenta (which is important in fetal metabolism) and the blood-brain barrier (which leads to irreversible neurologic damage), but not others· the liver and the kidneys (which are the normal selective barriers for bilirubin elimination and hence detoxification) [1-4,11] The ability to form intramolecular hydrogen bonds is thus an important determinant of conformation stabilization as well as the pigment's unusual polarity and solubility properties, all of which have important implications for biological function.

Collectively, three structural elements appear to have a dominating effect on the shape of bilirubin (1) two dipyrrinone chromophores, each in a *syn*-periplanar conformation with *Z*-configuration C=C bonds

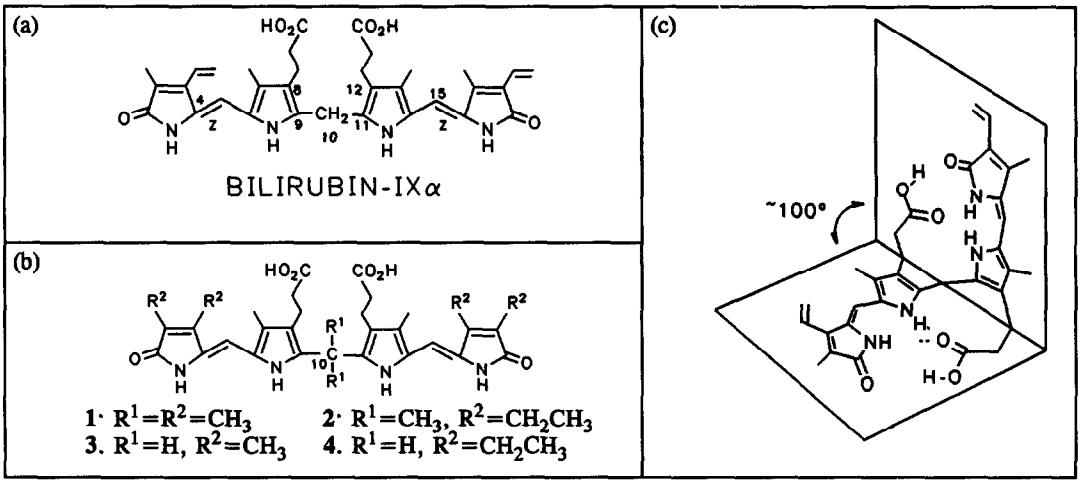
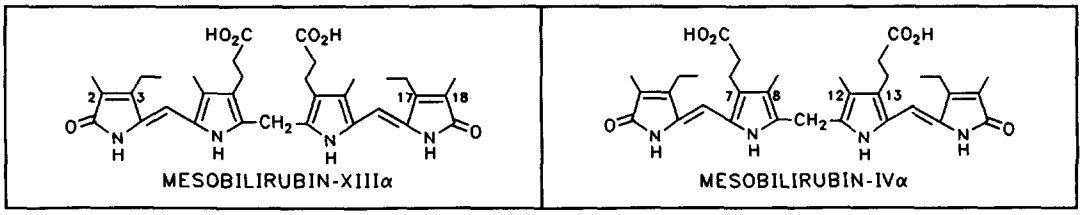


FIGURE 1. (a) Linear representations of (a) (4Z,15Z)-bilirubin-IXα and (b) 10,10-dimethylbilirubin analogs (**1** and **2**) and their parent rubins (**3** and **4**) (c) Ridge-tile intramolecularly hydrogen-bonded bilirubin

at C(4) and C(15); (ii) an sp^3 carbon at C(10), which constrains the molecule to bend in the middle and allows the two dipyrrinone chromophores to rotate independently about the C(9)-C(10) and C(10)-C-(11) single bonds, and (iii) two propionic acid groups, located at C(8) and C(12), which can form intramolecular hydrogen bonds with the pyrrole and lactam functions in the opposite half of the molecule (Figure 1c). Sequestration of the carboxylic acids through intramolecular hydrogen bonding lowers their acidity, increases the lipophilicity of the pigment and renders it unexcretable in normal metabolism, except by glucuronidation Structural modifications which do not interfere with this unique ability, *i e* , reduction of vinyl groups to ethyl or interchanging methyl and vinyl or ethyl groups at C(2)/C(3) or C(17)/C(18), *e g.*, mesobilirubin-XIIIα, afford bilirubin analogs with similar solubility properties soluble in chloroform, insoluble in methanol, insoluble in dilute aqueous bicarbonate Isomers that do not have their propionic acid groups located at C(8) and C(12), *e g* , mesobilirubin-IVα, are known to have very different solubility properties· insoluble in chloroform, soluble in methanol and soluble in dilute aqueous bicarbonate However, there are more subtle modifications which might be expected to disengage intramolecular hydrogen bonding without relocating the propionic acid groups These seemingly minor perturbations, created by intramolecular steric buttressing, might also be expected to exert a major influence on conformation and hence the properties of the pigment In the following we describe syntheses of two C(10)-dimethyl bilirubin analogs, 10,10-dimethylglaucorubin (**1**) and 10,10-dimethyl-2,18-diethylmesobilirubin-XIIIα (**2**) and compare their properties to those of their newly synthesized parent, bilirubin analogs, (**3**) and (**4**) (Figure 1)



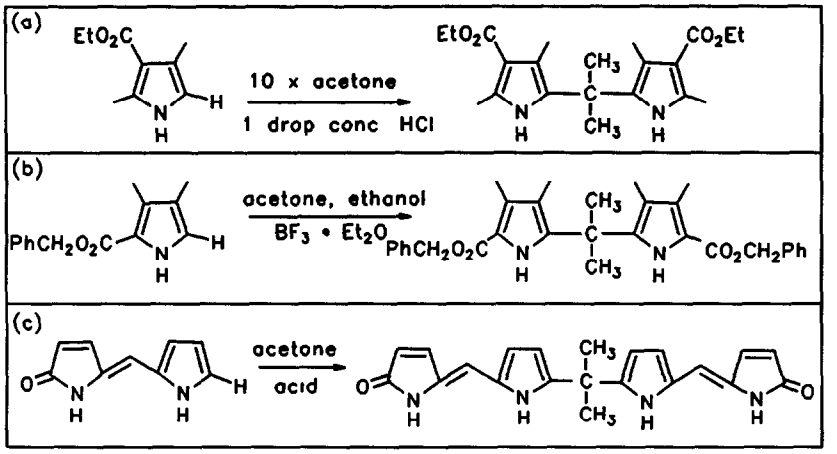


FIGURE 2. Syntheses of *gem*-dimethyldipyrrylmethanes according to (a) Fischer[12a] and (b) Smith [12b] (c) Proposed coupling of an α-unsubstituted generic dipyrrinone to give a 10,10-dimethylbilirubin analog

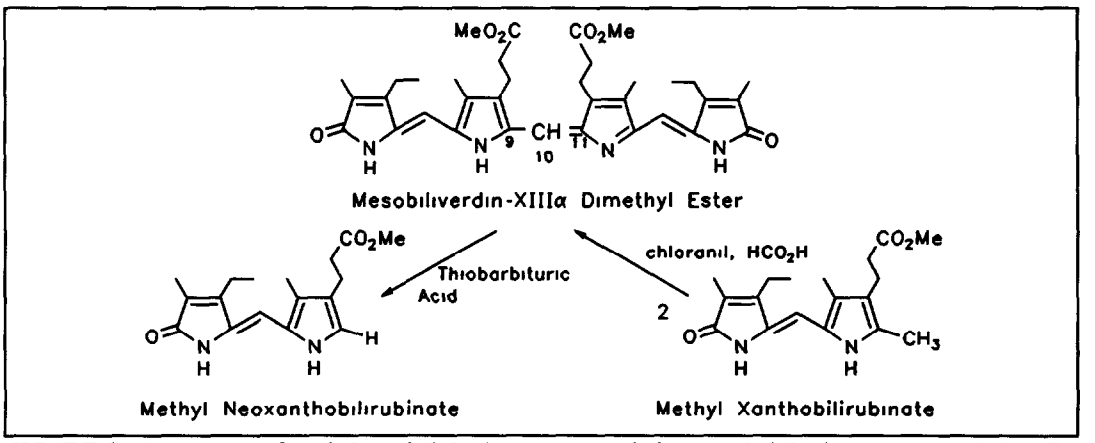


FIGURE 3. Conversion of methyl xanthobilirubinate into methyl neoxanthobilirubinate via mesobiliverdin-XIIIα dimethyl ester.

SYNTHESIS

Our initial approach toward the synthesis of a 10,10-dimethyl analog of bilirubin was based on the precedence, albeit limited, for coupling α-unsubstituted pyrroles with acetone to form *gem*-dimethyldipyrrylmethanes (Figure 2a and b) [12] Given a suitable α-unsubstituted dipyrrinone, it seemed reasonable to assume that condensation with acetone would afford the desired tetrapyrrole (Figure 2c) At that time the most readily available α-unsubstituted dipyrrinone was methyl neoxanthobilirubinate, which was obtained by treatment of mesobiliverdin-XIIIα dimethyl ester with thiobarbituric acid according to the procedure of Manitto and Monti (Figure 3) [13] Preparation of the verdin can be achieved by oxidative coupling of methyl xanthobilirubinate (available in a 10-step total synthesis)[14] or from catalytic hydrogenation then oxidation and esterification of bilirubin-XIIIα (available from bilirubin-IXα)[15] Since the verdin gives only one equi-

valent of methyl neoxanthobilirubinate (the second is a covalent adduct with thiobarbituric acid), this procedure can be seen as inefficient way to convert methylxanthobilirubinate into methyl neoxanthobilirubinate. A more direct route was seen as a modification of the final coupling step in the synthesis of methyl xanthobilirubinate[14] (Figure 4a) using opsopyrrole, its ethyl ester (**17**) or its tricarboxylic acid precursor (**19**) as the right half. Surprisingly, however, the starting materials were recovered unchanged. Despite this disappointment, a new modification of the synthesis of **20** was achieved by condensing ethyl 4-acetyl-5-oxohexanoate[14] with diethyl oximinomalonate[16] (rather than nitrosated ethyl acetoacetate)[14a], raising the yield from ~50% to ~75%. And an improved synthesis of opsopyrrole ethyl ester (**17**) was devised, overcoming the difficulties associated with the high temperature (autoclave) double decarboxylation of triacid **19**. Following highly selective esterification of **19** using ethyl orthoformate (giving essentially exclusively **18**), the monoester (**18**) was smoothly converted to opsopyrrole ethyl ester (**17**) in an overall 81% yield from **19** during (Kugelrohr) distillation at 2 mm Hg. This modification is much more efficient and convenient than the Fischer procedure used recently [17]

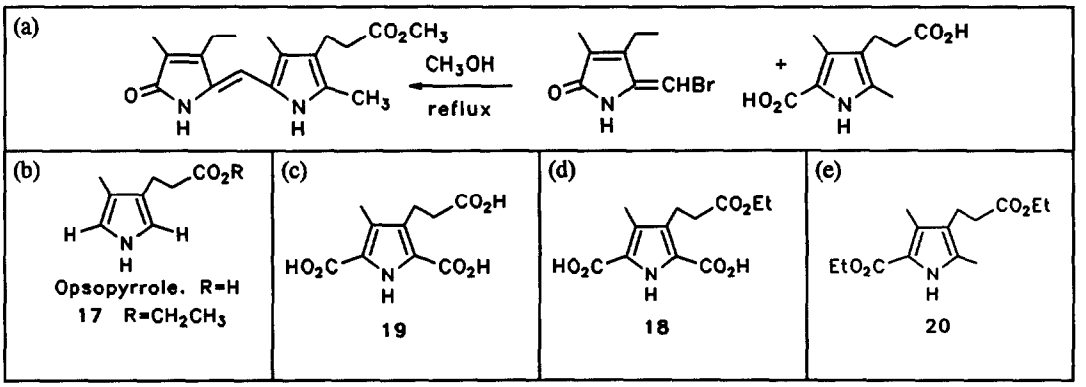
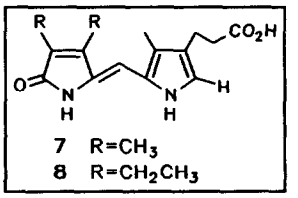
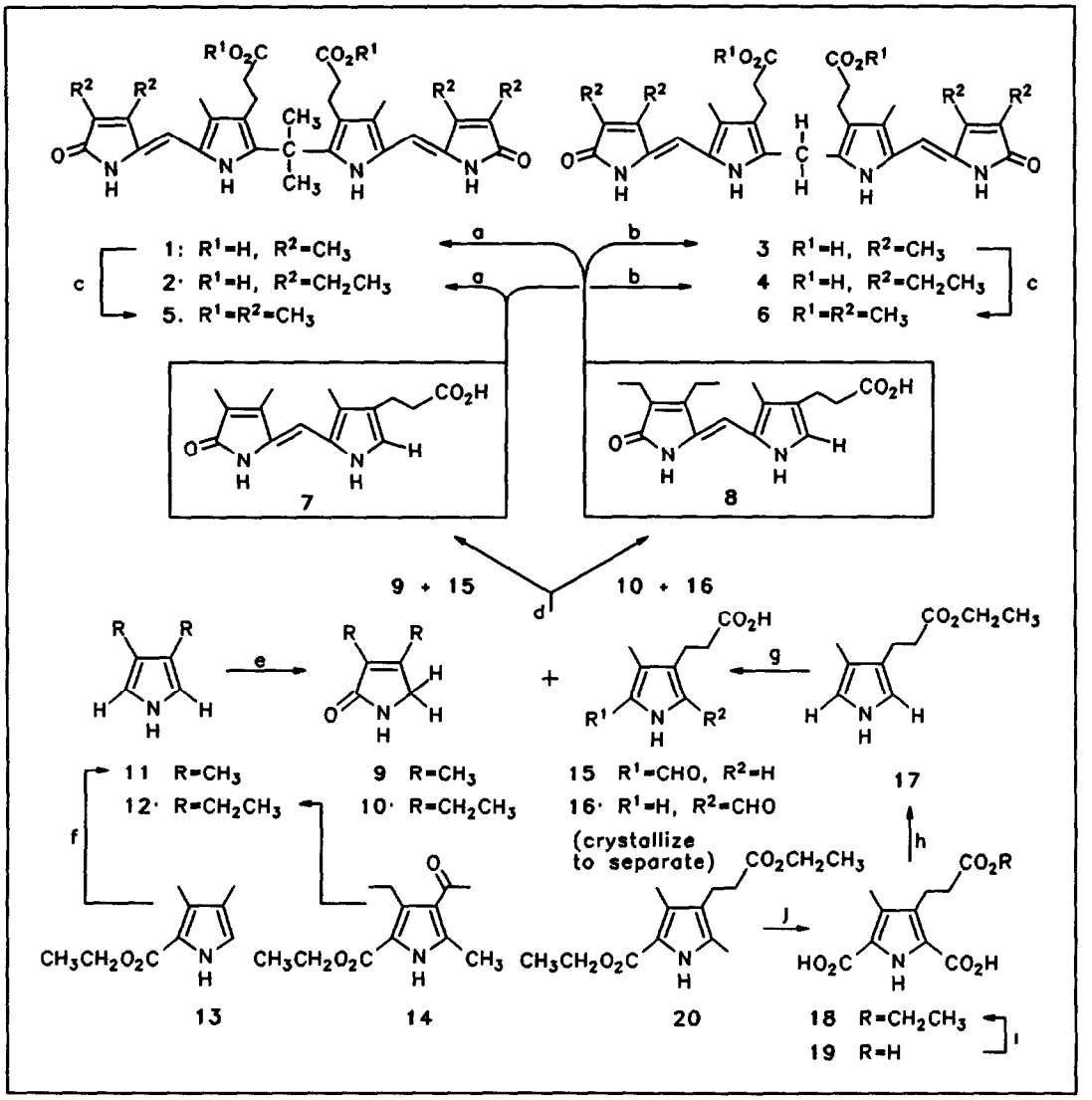


FIGURE 4. (a) Final coupling step in the synthesis[14a] of methyl xanthobilirubinate (b)-(e) Opsopyrrole and its synthetic precursors



Consequently, we directed our attention to the base-catalyzed condensation of a pyrrole α-aldehyde with a pyrrolenone[18] as a more direct total syntheses of α-unsubstituted dipyrrinones **7** and **8**, which differ from methyl neoxanthobilirubinate only by having either two methyl β-substituents in the lactam ring (**7**) or two ethyl β-substituents (**8**). The per-β-methylated analog was prepared by a similar route. As outlined in the Synthetic Scheme, the key step in the dipyrrinone (**7** and **8**) syntheses is base-catalyzed condensation of (the right half) pyrrole α-aldehyde **15** with the appropriate (left half) pyrrolinone, either **9** or **10** The yields in this step are typically quite high, e.g , 90% yield of **7** on a 10-gram scale from **9** and **15** The important intermediate (**15**) was formed in a Vilsmeier reaction[17] on opsopyrrole ethyl ester (**17**) along with its isomer (**16**) and separated by crystallization. Although the Vilsmeier reaction gave an 80% yield of a 4.1 mixture of **15** **16**, the major isomer (**15**) is much less soluble in methanol (less polar due to intramolecular hydrogen bonding between the CO_2H and CHO groups?) This facilitated removing the minor isomer (**16**) by washing or crystallization

SYNTHETIC SCHEME



[a] $(CH_3)_2C(OCH_3)_2/CF_3CO_2H$, [b] CH_2O/HCl, [c] carbonyldiimidazole, then CH_3OH, [d] KOH/CH_3OH, [e] H_2O_2, [f] NaOH, then HCl, Δ; [g] Vilsmeier, [h] Δ, distil, [i] $HC(OCH_2CH_3)_3/CF_3CO_2H$, [j] SO_2Cl_2, then NaOH

The final step, converting an α-unsubstituted dipyrrinone to a 10,10-dimethylbilirubin, proved tricky Treating methyl neoxanthobilirubinate (or the methyl ester of **7**) in acetone solvent with acid (*p*-toluenesulfonic acid, trifluoroacetic acid (TFA) gave no tetrapyrrole, only the α-isopropenyl derivative. The latter would not undergo further (acid-catalyzed) reaction with starting dipyrrinone. Even when the reactant was changed to 2,2-dimethoxypropane and the solvent changed to TFA, the same result obtained However,

when dipyrrinone *acid* **7** was reacted with 2,2-dimethoxypropane in TFA for 5 minutes, followed by quenching in ice water, a new yellow product was obtained along with unreacted starting dipyrrinone. It was much less polar than starting dipyrrinone acid, soluble in chloroform and extractable from chloroform into 5% aqueous sodium bicarbonate The structure was confirmed as the desired 10,10-dimethylbilirubin analog (**1** or **2**) by spectroscopic methods Reaction of **7** with 38% aq. formaldehyde in conc. hydrochloric acid for 5 minutes led smoothly to bilirubin analog **3**, glaucorubin, which is also less polar than starting dipyrrinone. Similarly, reaction of **8** with 2,2-dimethoxypropane in TFA gave 10,10-dimethyl bilirubin analog **2**, and reaction with formaldehyde gave bilirubin analog **4**

En route to this collection of tetrapyrroles, we improved the yields of several synthetic intermediates Ester **13** was prepared in an improved yield, then it was saponified, dried and decarboxylated during distillation (Kugelrohr) under a water aspirator pressure at 180°C to afford pure crystalline 3,4-dimethyl pyrrole (**11**) in 89% yield. Oxidation of **11** to pyrrolinone **9** (or **12** to **10**) was improved by using a short reflux time in pyridine-methanol (15 1) with excess 30% hydrogen peroxide to afford product in 90% isolated yield.

A direct route to the preparation of dimethyl ester (**5**) by treating the methyl ester of **7** with 2,2-dimethoxypropane and various acid catalysts was unsuccessful The coupling reaction was confirmed to stop at an intermediate stage, where the free α-position is substituted by an isopropenyl group. However, the esterification of rubin was smoothly accomplished by activation of the acid functional group with carbonyl diimidazole then treatment with methanol.[19] The corresponding dimethyl ester (**6**) of (**3**) was also prepared in a similar way

RESULTS AND DISCUSSION

Consistent with their C(10) *gem*-dimethyl structures and in contrast to bilirubin, **1** and **2** are not sensitive to oxidation to verdins Consequently, they are easily handled and purified They also differ in solubility properties from their parent pigments (**3** and **4**), which exhibit properties typical of bilirubin and mesobilirubin XIII. Significantly, **1** and **2** are soluble in methanol, but **3** and **4** are insoluble, **1** and **2** are soluble in 5% aq sodium bicarbonate, but **3** and **4** are insoluble; and, although **1**, **2**, **3** and **4** are all soluble in chloroform, the solubility of **1** and **2** is about 10 times greater than their corresponding parents, **3** and **4** The behavior of **1** and **2** is curious because they appear to be more polar than their parents in the reverse phase HPLC chromatography system developed by McDonagh[20] (0.1 *M* di-n-octylamine acetate, in methanol, pH 7 7, 3% H_2O) Thus, although **1** (retention time 5 72 min) moves 2 min slower than its dipyrrinone precursor (**9**) as expected, it moves 2 min. *faster* than its parent (**3**), which exhibits a retention time (7 65 min) characteristic of intramolecularly hydrogen bonded bilirubins The results suggest that amphiphilic pigments **1** and **2** possess structures different from **3** and **4**.

The constitutional structures of the C(10) *gem*-dimethylbilirubin analogs are consistent with their ^{13}C-NMR (Table 1) Thus, for 10,10-dimethylglaucorubin (**1**) and its 2,3,17,18-tetraethyl analog (**2**) and the dimethyl ester of **1** (**5**), one finds the expected carbon chemical shifts of ring carbon and β-substituents of the parent glaucorubin (**3**), the additional CH_3 resonances near $\delta=29$ for the *gem*-dimethyls attached to C(10), and a deshielding of C(10) from $\delta\simeq24$ to $\delta\simeq37$ The spectra are in complete accord with our expectations for the assigned structures (Figure 1). In $CDCl_3$ solvent the ^{13}C-NMR spectra of **1**, **2** and **5** were also very similar; however, in this solvent the quaternary C(10) resonance is shifted to $\delta\simeq42$ in the

Table 1. ^{13}C-NMR Chemical Shifts and Assignments for 10,10,Dimethylglaucorubin (1), Glaucorubin (3), Dimethyl Esters (5) and (6) and 10,10-Dimethyl-2,18-diethylmesobilirubin-XIIIα (2) in $(CD_3)_2SO$[a]

Position[b]	Carbon	δ for 1	δ for 2	δ for 3	δ for 5	δ for 6
1,19	C=O	174 05	174 12	174 03	171 88	172 13
2,18	=C—	123 19	123 25	122 54	123 15	123 17
2^1,18^1	CH_3/CH_2[c]	8 40	17 52	8 10	8 49	8 17
2^2,18^2	CH_3	—	15 72	—	—	—
3,17	=C—	141 60	141 78	147 23	142 12	140 95
3^1,17^1	CH_3/CH_2[c]	9 35	19 87	9 40	9 47	9 59
3^2,17^2	CH_3	—	16 56	—	—	—
4,16	=C—	129 96	130 04	130.38	130 02	130 33
5,15	=CH—	98 31	98 40	97 80	98 41	97 94
6,14	=C—	124 22	124 32	122 00	124 58	123 51
7,13	=C—	121 74	121 56	122 90	122 11	122 75
7^1,13^1	CH_3	9 64	10 35	9 25	9 58	9 25
8,12	=C—	118 50	118 55	119 23	118 81	118 97
8^1,12^1	CH_2	19 81	19 87	19 27	19 85	19 34
8^2,12^2	CH_2	34 32	34 29	34 34	34 44	34 12
8^3,12^3	C=O	172 43	172 58	172 00	173 19	173 31
8^4,12^4	OCH_3	—	—	—	50 95	50 91
9,11	=C—	138 63	138 73	128 81	138 78	130 57
10		36 59	36 65	23 55	36 77	23 71
10^1	CH_3	29 07	29 12	—	29 05	—

[a] Run at 2 5 x 10^{-2} M concentration of pigment at 22°C with chemical shifts recorded in ppm downfield from $(CH_3)_4Si$ Multiplicites are determined by the APT method [b] Superscripts refer to carbons in the β-substituent chains, e g 2^1 is the first carbon attached to ring carbon C(2)

acids but remains near δ≃36 in the dimethyl ester We surmise that the deshielding is associated with the formation of intramolecular hydrogen bonds in 1 and 2 (but not in their dimethyl esters) In support of this, the analog of 1 with methyl groups at all pyrrole β-positions has δ≃36 for C(10) in the ^{13}C-NMR in $CDCl_3$ In contrast, no similarly large shift is observed in the parent acid (3) and its dimethyl ester (4). More direct evidence for (conformation-determining) intramolecular hydrogen bonding comes from ^{1}H-NMR spectroscopy

^{1}H-NMR spectroscopy clearly reveals intramolecular hydrogen bonding between the CO_2H and lactam -NH-C=O groups in the new bilirubins in $CDCl_3$, a solvent that preserves intramolecular hydrogen bonding[8,22] (Table 2) Although the CO_2H and lactam NH resonances are significantly deshielded due to

hydrogen bonding, the ^{1}H-NMR spectra of **1** and **3** or **2** and **4** also show important differences in the NH region As expected, both the lactam and pyrrole NH resonances of **3** and **4** are at almost the same chemical shift as those of mesobilirubin-XIIIα (δ 10 61 and 9.15), which is thought to adopt the ridge-tile intramolecularly hydrogen bonded conformation (Figure 1). In contrast, the *gem*-dimethyl analogs (**1**) and (**2**) lactam N-*H* resonances are more strongly deshielded, falling at the lowest field observed among all known rubin acids and the pyrrole N-*H* signals of **1** and **2** fall at the highest field (δ 8 91-8.94) observed. The data for **1** and **2** suggest stronger hydrogen bonding between the CO_2H and the lactam -NH-C=O and less effective intramolecular hydrogen bonding to the pyrrole N-H Presumably, steric interactions between the *gem* dimethyls at C(10) cause flattening of the ridge-tile conformation and loosening of the intramolecular hydrogen bonding matrix, forcing the propionic CO_2H group to disengage from the pyrrole N-H somewhat In this more opened ridge-tile conformation, the propionic acid groups engage in stronger hydrogen bonds with the lactam C=O and NH groups, leading to stronger deshielding in both lactam NH signal and in the CO_2H signal, *cf* **3** and **4**. On the basis of the data, we suggest that **1-4** have a conformation in chloroform similar to that shown for bilirubin in Figure 1, but the conformations of **1** and **2** differ from **3** and **4** in tending to have a more open ridge-tile shape.

TABLE 2 ^{1}H-NMR Chemical Shifts, Multiplicities and Assignments for 10,10-Dimethylglaucorubin (**1**), 10,10-Dimethyl-2,18-diethylmesobilirubin-XIIIα (**2**), Glaucorubin (**3**) and 2,18-Diethylmesobilirubin-XIIIα (**4**) in $CDCl_3$ [a]

Position	Proton	δ for **1**	δ for **3**	δ for **2**	δ for **4**
8^3,12^3	CO_2H	13 93 (s)	13 59 (s)	14 10 (s)	13 67 (s)
21,24	NH	11 08 (s)	10 61 (s)	11 10 (s)	10 59 (s)
22,23	NH	8 91 (s)	9 15 (s)	8 94 (s)	9 14 (s)
5,15	=CH—	6 02 (s)	6 04 (s)	6 04 (s)	6 04 (s)
10	CH_2	—	4 07 (s)	—	4 09 (s)
10^1	$C(CH_3)_2$	2 07 (s)	—	2 07 (s)	—
8^1,12^2	CH_2	2 55-3 50 (m)	2 50-3 00 (m)	2 50-3 50 (m)	2 50-3 00 (m)
8^2,12^2	CH_2	2 55-3 50 (m)	2 50-3 00 (m)	2 50-3 50 (m)	2 50-3 00 (m)
7^1,13^1	CH_3	2 14 (s)	2 16 (s)	2 14 (s)	2 16 (s)
3^1,17^1	CH_3 or CH_2	2 06 (s)	2 06 (s)	2 48 (q)[b]	2 47 (q)[b]
3^2,17^2	CH_3	—	—	1 15 (t)[b]	1 15 (t)[b]
2^1,18^1	CH_3 or CH_2	1 85 (s)	1 85 (s)	2 31 (q)[b]	2 30 (q)[b]
2^2,18^2	CH_3	—	—	1.08 (t)[b]	1 10 (t)[b]

[a] Run at 4 x 10^{-3} *M* concentration of pigment at 22°C with chemical shifts recorded in ppm downfield from $(CH_3)_4Si$ [b] J=7 5 Hz

The ^{1}H-NMR data in $CDCl_3$ may be compared with those in $(CD_3)_2SO$ (Table 3). The spectra of (**1**), (**3**) and their 2,3,17,18-tetraethyl analogs (**2**) and (**4**) are essentially identical, differing significantly only in that the 10,10-dimethyl pigments exhibit new methyl resonances at 1 73 δ, which replace the

characteristic C(10) CH_2 signals at 3.95 δ (*cf* **1** and **3**, **2** and **4**). In $(CD_3)_2SO$, the NH and CO_2H chemical shifts are governed by association with the solvent.[21] The somewhat shielded NH chemical shifts of **1** and **2** relative to **3** and **4** suggest weaker hydrogen bonds to sulfoxide in the former, possibly as a result of an altered conformation due to the *gem*-dimethyl effect Since the conformation of bilirubin in $(CD_3)_2SO$ is believed to be one where the propionic acid residues are tied to bound solvent molecules with loose but unspecified hydrogen-bonding,[8,22,23] the conformation of **1** and **2** may be similar These observations are fully consistent with the constitutional structural differences concluded by ^{13}C-NMR spectroscopy

TABLE 3 ^1H-NMR Chemical Shifts, Multiplicities and Assignments for 10,10-Dimethylglaucorubin (**1**), Glaucorubin (**3**), 10,10-Dimethyl-2,18-diethylmesobilirubin-XIIIα (**2**) and 2,18-Diethylmesobilirubin-XIIIα (**4**) in $(CD_3)_2SO$ [a]

Position	Proton	δ for **1**	δ for **3**	δ for **2**	δ for **4**
$8^3,12^3$	CO_2H	11 90 (brs)	11 89 (brs)	11 89 (brs)	11 90 (brs)
21,24	NH	9 48 (brs)	9 78 (brs)	9 49 (brs)	9 78 (brs)
22,23	NH	10 13 (brs)	10 32(brs)	10 13 (brs)	10 32 (brs)
5,15	=CH-	5 95 (s)	5 94 (s)	5 95 (s)	5 94 (s)
10	CH_2	—	3 95 (s)	—	3 95 (s)
10	$C(CH_3)_2$	1 73 (s)	—	1 73 (s)	—
$8^1,12^2$	CH_2	2 19 (t)[b]	2 41 (t)[b]	2 20 (t)[c]	2 40 (t)[c]
$8^2,12^2$	CH_2	1 98 (t)[b]	1 92 (t)[b]	1 99 (t)[c]	1 93 (t)[c]
$7^1,13^1$	CH_3	2 06 (s)	2 05 (s)	2 05 (s)	2 06 (s)
$3^1,17^1$	CH_3 or CH_2[d]	1 96 (s)	2 00 (s)	2 46 (q)[c]	2 45 (q)[c]
$3^2,17^2$	CH_3	—	—	1 09 (t)[c]	1 08 (t)[c]
$2^1,18^1$	CH_3 or CH_2	1 77 (s)	1 77 (s)	2 26 (q)[c]	2 25 (q)[c]
$2^2,18^2$	CH_3	—	—	1 04 (t)[c]	1 05 (t)[c]

[a] Run at 4 x 10^{-3} *M* concentration of pigment at 22°C with chemical shifts recorded in ppm downfield from $(CH_3)_4Si$ [b] J=7 8Hz [c] J=7 5 Hz [d] CH_3 in **1** and **2**, CH_2 in **3** and **4**

Information on conformation of the dimethyl esters can be extracted from their ^1H-NMR spectra The chemical shifts of Table 4 reveal significant differences between dimethyl 10,10-dimethylglaucorubinate (**5**) and dimethyl glaucorubinate (**6**) In particular, the NH resonances of **5** are more shielded than those of its parent **6** in $CDCl_3$ and $(CD_3)_2SO$, suggesting different conformations Ester **6** exhibits pyrrole and lactam N-*H* chemical shifts typical of mesobilirubin-XIIIα dimethyl ester (δ= 10 28 and 10 21, $CDCl_3$), and since the latter is known to self-associate through *inter*molecularly hydrogen bonds and adopt a porphyrin-like conformation in non-polar solvents,[5] we assume that **6** does likewise In contrast, ester **5** has significantly more shielded NH resonances than **6** Thus it would seem that the two methyl groups at C(10) defeat the ability of **5** to participate as easily in intermolecular hydrogen-bonding, presumably by altering the conformation of the *gem*-dimethyl rubin ester relative to the parent

TABLE 4 ^{1}H-NMR Chemical Shifts and Assignments for the Dimethyl Esters of 10,10-Dimethylglaucorubin and Glaucorubin (**5**) and (**6**) in $CDCl_3$ solvents [a]

Position	Proton	δ for **5** in $(CD_3)_2SO$	δ for **6** in $(CD_3)_2SO$	δ for **5** in $CDCl_3$	δ for **6** in $CDCl_3$
21,24	NH	9 51 (brs)	9 78 (brs)	9 71 (s)	10 44 (s)
22,23	NH	10 12 (brs)	10 41 (brs)	8 71 (s)	10 28 (s)
5,15	=CH-	6 01 (s)	5 92 (s)	5 96 (s)	5 98 (s)
10	CH_2	—	4 13 (s)	—	4 05 (s)
10	$C(CH_3)_2$	1 84 (s)	—	1 72 (s)	—
8^1,12^2	CH_2	2 45 (t)[b]	2 88 (t)[b]	2 50 (t)[b]	2 52 (t)[b]
8^2,12^2	CH_2	2 22 (t)[b]	2 49 (t)[b]	2 22 (t)[b]	2 25 (t)[b]
7^1,13^1	CH_3	2 14 (s)	2 16 (s)	2 05 (s)	2 06 (s)
3^1,17^1	CH_3	2 04 (s)	1 93 (s)	1 94 (s)	1 95 (s)
3^2,17^2	CH_3	1 09 (t)[b]	1 08 (t)[b]	1 15 (t)[b]	1 15 (t)[b]
2^1,18^1	CH_3	1 89 (s)	1 54 (s)	1 76 (s)	1 77 (s)
2^2,18^2	CH_3				
8^4,12^4	OCH_3	3 47 (s)	3 52 (s)	3 47 (s)	3 52 (s)

[a] Run at 4 x 10^{-3} *M* concentration of pigment at 22°C with chemical shifts recorded in ppm downfield from $(CH_3)_4Si$ [b] J=7 5 Hz

Further evidence on the question of *inter*molecularly H-bonded dimeric conformations in esters **5** and **6** comes from solvent-dependent UV-visible spectra (Table 5) Surprisingly, but as found for the *gem*-dimethyl parent acid (**1**), the UV-visible spectral λ^{max} of ester **5** changes little in going from non-polar to polar solvents, *e g.*, from benzene to chloroform to methanol to dimethylsulfoxide This behavior was unexpected and contrasts with the more normal solvent dependence of ester **6**, which is quite similar to that

TABLE 5. UV-Visible Spectroscopic Data [ϵ^{max} (λ^{max})] for 10,10-Dimethylglaucorubin (**1**), Glaucorubin (**3**) and Their Dimethyl Esters (**5**) and (**6**)

Solvent	**1**	**3**	**5**	**6**
C_6H_6	50600(434)	Insol	36000(404)	52700(384)
CH_2Cl_2	50100(425)	51000(427)	40500(414)	57600(379)
$CHCl_3$	50400(434)	58000(431)	40700(414)	48400(381) 36600(411)[sh]
$(CH_3)_2CO$	46300(426)	Insol	40900(413)	47000(381) 34000(427)[sh]
CH_3OH	46000(426)	Insol	46400(424)	47400(427)
$(CH_3)_2SO$	58000(425) 44200(395)[sh]	57300(426)	45500(420)	43500(428) 32500(389)[sh]

of mesobilirubin-XIIIα dimethyl ester, an ester which prefers to dimerize through *inter*molecular hydrogen bonding in non-polar solvents such as benzene and chloroform while remaining monomeric in polar solvents such as methanol and dimethyl sulfoxide. On the basis of exciton coupling analyses,[24] the pigment structure in the dimer is thought to be porphyrin-like, and in the monomer more open, as in a ridge-tile conformation The data of Table 5 suggest that the C(10) *gem*-dimethyl discourages easy (associative) dimer formation in **5**, leaving monomeric, possibly weakly intramolecularly hydrogen bonded conformations in non-polar solvents, consistent with earlier molecular modelling analyses of bilirubin dimethyl ester [5]

CONCLUDING COMMENTS

For the first time bilirubin derivatives have been synthesized with a *gem*-dimethyl group at C(10) Addition of the methyl groups introduces an internal steric buttressing at the propionic acid chains that somewhat destabilizes the original intramolecularly hydrogen bonded ridge-tile conformation, rendering gem-dimethyl congeners **1** and **2** amphiphilic, more soluble in both polar and non-polar organic solvents than the parent pigments **3** and **4** or bilirubin, and also more soluble in dilute bicarbonate. These results are important because they reveal a new way to alter the stereochemistry and hence the solution properties of intramolecularly hydrogen-bonded bilirubin pigments by judiciously targeting remote substitution. They also provide new bilirubin pigments, of which there are no verdin analogs, for use in establishing the role of hydrogen-bonding in protein binding, transport and metabolism studies

EXPERIMENTAL

General Procedures All ultraviolet-visible spectra were recorded on a Perkin-Elmer 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 Analytical thin layer chromatography was carried out on J.T Baker silica gel IB-F plates (125 μ layers) 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 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 3% aqueous methanol (pH 7 7, 31°C).

Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). 2-Butanone, ethyl formate, diethylmalonate, sulfuryl chloride, trifluoroacetic acid, 38% aq formaldehyde, acetic acid, tetrahydrofuran, N,N-dimethylformamide, phosphorous oxychloride, acetonitrile, and dimethylsulfoxide were from Aldrich Tetrahydrofuran was dried by distillation from sodium

2-Carboethoxy-3,4-dimethyl-1*H*-pyrrole (13). Forty-six grams (2.0 g atoms) of freshly cut sodium was added to a solution of 150 g (2 08 moles) of methyl ethyl ketone and 150 g (1 97 moles) of ethyl formate in anhydrous ether (2 L) with mechanical stirring over a period of 4 hours, during which time the mixture was chilled in an ice-salt bath The mixture was then stirred for 14 hours at room temperature The solvent was evaporated and to the residue (sodium salt of 2-methyl-3-oxobutyraldehyde) was added glacial acetic acid (870 mL), anhydrous sodium acetate (240 g), and diethyloximinomalonate (285 g, 1 51 moles). The

mixture was heated to 90°C, then zinc dust (310 g, 4 74 g-atoms) was added in small portions such that the pot temperature of the reaction can be controlled between 95-100°C by air cooling. After the addition of zinc, the reaction mixture was heated at reflux for two hours The resulting hot, light brown liquid was poured into 2 L of ice water and allowed to stand in the cold room overnight The slightly yellow precipitate was collected by filtration, washed with water and dried by suction. The crude solid was dissolved in hot hexane-ethyl acetate and cooled to give 131 g of light yellow crystalline pyrrole (**8**) in 51% yield It had mp 91-92°C (Lit.[13(a)] mp 90-91°C); IR (deposition from CH_2Cl_2) ν· 3320, 2925, 1732, 1720, 1695, 1675, 1660, 1465 cm^{-1}, ^{1}H-NMR δ· 1.32 (t, 3H, J=7 2 Hz), 2.01 (s, 3H), 2 24 (s, 3H), 4 28 (q, 2H, J=7 2 Hz), 6 64 (s, 1H), 8.71 (broad, NH) ppm, ^{13}C-NMR δ: 9 85 (q), 10 21 (q), 14.50 (q), 59 75 (q), 119.34 (d), 120 05 (s), 120 55 (s), 126.53 (s), 161 75 (s) ppm

3,4-Dimethyl-1*H*-pyrrole (11). Pyrrole **13** (20 g, 120 mmole) was suspended in 20 mL of 95% ethanol and 15 g of sodium hydroxide in 150 mL water, and the mixture was heated at reflux for 3 hours The resulting yellowish solution was evaporated to remove the alcohol, then the remaining solution was diluted to 200 mL with water and acidified with 28 mL of acetic acid under cooling to give a white precipitate The fine white solid was collected, washed with water, dried and decarboxylated during distillation at 180°C in a Kugelrohr apparatus at water aspirator pressure to give crystalline 3,4-dimethylpyrrole, mp 30-31°C (Lit.[13] mp 30-31°C), 10 2 g, 89% yield It had ^{1}H-NMR ($CDCl_3$) δ 2 03 (s, 6H), 6 52 (d, 2H, J=2 4 Hz), 7 76 (broad, NH) ppm; ^{13}C-NMR δ 9 94 (q), 115 81 (d), 118 18 (s) ppm

3,4-Dimethyl-3-pyrrolin-2-one (9). 3,4-Dimethylpyrrole (**11**) (9 5 g, 0 1 mole) in 15 mL of dry pyridine and 1 mL of methanol containing 15 mL of 30% H_2O_2 was heated carefully at gentle reflux for 10 min Then another 5 mL of 30% H_2O_2 was added and heating was continued at reflux for 15 min more. At this time, almost all of the starting pyrrole had been converted to oxopyrrole, as confirmed by GC-MS The solvents were evaporated at 50°C, affording yellowish oily product The oily residue was taken up in 100 mL of dichloromethane, was washed with water (50 mL x 4), dried and evaporated, leaving a slightly yellow solid after cooling, 10 8 g of dried oxopyrrole in the yield of 95% This crude product was of sufficient purity for the next step It had mp 93-95°C (Lit.[13] no mp reported), ^{1}H-NMR δ 1 74 (s, 3H), 1 94 (s, 3H), 3.77 (s, 2H), 7.65 (broad, NH) ppm, ^{13}C-NMR δ· 8 18 (q), 13 25 (q), 50 29 (t), 128 30 (s), 149 35 (s), 176 80 (s) ppm

2,4-Dimethyl-5-(ethoxycarbonyl)-1*H*-pyrrole-3-propanoic acid ethyl ester (20). To a 5 L three-neck round bottom flask equipped with a mechanical stirrer, dropping funnel and thermometer, were added ethyl 4-acetyl-5-oxohexanoate[14] (84 g, 0.42 mole), diethyloximinomalonate[16] (83 g, 0 43 mole), anhydrous sodium acetate (105 g, 1 28 mole) and 425 mL glacial acetic acid. The mixture was heated to 90°C, then zinc dust (93 g, 1 43 g-atom) was added in small portions to the vigorously stirred solution such that the pot temperature did not exceed 100°C (controlled by air cooling). When the zinc had been added completely, the reaction mixture was heated with stirring to reflux for two hours. The hot, light brown liquid was poured into 2 L of ice water and allowed to stand in the cold room overnight The resulting white precipitate was collected by filtration and washed with water; then it was dissolved in dichloromethane and filtered The clear and dried solution was evaporated to remove the solvent under vacuum to afford 83 g of fairly pure white product in 75% yield It had mp 72-73°C (Lit.[14] mp 72-73°C), ^{1}H-NMR δ 1 18 (t, 3H, J=7.2 Hz), 1 31 (t, 3H, J=7.2 Hz), 2.18 (s, 3H), 2.22 (s, 3H), 2 38 (t, 2H, J=7 0 Hz), 2 68 (t, 2H, J=7 0 Hz), 4.08 (q, 2H, J=7 2 Hz), 4 26 (q, 2H, J=7 2 Hz), 7 86 (broad, NH) ppm, ^{13}C-NMR δ 10 58 (q), 11 18 (q), 14 15 (q), 14 50 (q), 19 55 (t), 36 15 (t), 60 00 (t), 60 39 (t), 116 83 (s), 119 88 (s), 126 59 (s), 130 88 (s), 162 05 (s), 173.00 (s) ppm

4-Methyl-2,5-dicarboxy-1*H*-pyrrole-3-propanoic acid (19). To a solution of pyrrole diester **20** (26 7 g, 0 1 mole) in dry tetrahydrofuran (200 mL) was added freshly distilled sulfuryl chloride (40 5 g, 0 3 mole) dropwise at -15°C with stirring during 1 hour, giving a clear yellow solution Stirring was continued for 1 hour more at -15°C and then for 4 hours at 0°C The reaction solution was treated with 60 mL of water at 0-5°C and allowed to stand at room temperature overnight under vigorous stirring The solvent was removed under vacuum and the pale yellow precipitate was collected by filtration and washed with water The crude product was directly suspended in 10% aqueous sodium hydroxide solution (80 mL), and the

mixture was heated at reflux for 3 hours. The cooled brown solution was acidified with concentrated hydrochloric acid to pH 3, and the resulting precipitate was filtered and dried to give 21 g triacid-pyrrole (**19**) (yield 90%). It had mp 166°C (dec.). IR (KBr film) ν: 3365, 2958, 1671, 1655, 1527 cm^{-1}; ^{1}H-NMR ($CD_3)_2SO$) δ. 2.13 (s, 3H), 2.20 (t, 2H, J=7.2 Hz), 2.75 (t, 2H, J=7.2 Hz), 10.72 (broad, NH), 12 00 (broad, COOH) ppm. ^{13}C-NMR ($CD_3)_2SO$) δ: 10.14 (q), 20 31 (t), 35.16 (t), 122.78 (s), 125 64 (s), 129.16 (s), 162.68 (s), 174 56 (s) ppm

2,5-Dicarboxy-3-methyl-1*H*-pyrrole-4-propanoic acid ethyl ester (18). To pyrrole triacid **12** (31 g, 0 12 mole) in 150 mL of absolute ethanol was added 15 mL of trifluoroacetic acid with stirring. The mixture was stirred overnight; then 18 mL of triethyl orthoformate was added to the reaction and stirring was continued for 48 hours at room temperature The solvent was evaporated to give a red colored powder of the expected product in 97%. It had mp 210°C (dec); IR (KBr film) ν 3365, 1676, 1580, 1525 cm^{-1}, ^{1}H-NMR δ 1 35 (t, 3H, J=7 2 Hz), 2 39 (s, 3H), 2.73 (t, 2H, J=7 5), 3 19 (t, 2H, J=7.5 Hz), 4 29 (q, 2H, J=7.2 Hz), 9.81 (broad, NH) ppm; ^{13}C-NMR δ 9.50 (q), 13.29 (q), 19.94 (t), 34.70 (t), 62 92 (t), 121 62 (s), 121.95 (s), 130.46 (s), 131.91 (s), 165.70 (s), 166 13 (s), 177.45 (s) ppm. The compound was unstable and was used directly in the next step.

Ethyl-4-methyl-1*H*-pyrrole-3-propanoate (17). Decarboxylative-distillation of pyrrole monoester diacid **18** (18 7 g, 0.07 mole) was carried out in a kugelrohr apparatus at 180°C under a vacuum of 2 mm Hg during 5 hours This gave 10.2 g of liquid pyrrole product 81% yield It had bp 150-152°C/1 mm Hg (Lit.[16] bp 100-101°C/0 04 mm Hg); IR (deposition from CH_2Cl_2) ν 1725, 1686 cm^{-1}; ^{1}H-NMR δ 1 29 (t, 3H, J=7.2 Hz), 2.09 (s, 3H), 2.61 (t, 2H, J=7 5 Hz), 2.81 (t, 2H, J=7 5 Hz), 4 14 (q, 2H, J=7 2 Hz), 6 53 (d, 2H, J=2.5 Hz), 8 01 (broad, NH) ppm; ^{13}C-NMR δ· 10 67 (q), 14.89 (q), 21 48 (t), 35.86 (t), 60 97 (t), 115.80 (d), 116.53 (d), 118 00 (s), 122 02 (s), 174 36 (s) ppm

5-Formyl-4-methyl-1*H*-pyrrole-3-propanoic acid (15). Opsopyrrole ethyl ester (**17**) (10 g, 55 mmole) in 200 mL of dry absolute diethyl ether containing 5 g of N,N-dimethylformamide was added 10 g of posphorous oxychloride ($POCl_3$) dropwise with continuous stirring and cooling The reaction mixture was kept at room temperature overnight. Then the solvent was evaporated, leaving a dark oily product. To this was first added 120 mL of water, then 16 g of sodium hydroxide in 50 mL water under cooling The resulting mixture was heated almost to reflux for 30 min, then carefully acidified with concentrated hydrochloric acid at 0°C. The yellowish crystalline precipitate was collected and dried to give 8 1 g of product, 81% yield ^{1}H-NMR showed that the product contained both the 5-formyl isomer (**15**) and the 2-formyl isomer (**16**) in 4 1 ratio The predominant, more crystalline 5-formyl isomer was separated readily and with excellent recovery recrystallization in methanol It had mp 151-153°C (Lit [17] mp 154-155°C), ^{1}H-NMR δ 2 37 (s, 3H), 2 73 (t, 2H, J=7 2 Hz), 2 84 (t, 2H, J=7.2 Hz), 7.26 (s, 1H), 9 20 (s, 1H), 10.32 (broad, NH) ppm, ^{13}C-NMR δ· 8 82 (q), 19 36 (t), 34 15 (t), 125 69 (d), 128 74 (s), 131 35 (s), 138 58 (s), 176 59 (s), 180 16 (d) ppm The 2-formyl isomer (**16**) had mp 125-126°C (Lit [16] mp 125°C), ^{1}H-NMR δ 2 06 (s, 3H), 2 78 (t, 2H, J=7 5 Hz), 3 06 (t, 3H, J=7 5 Hz), 6 94 (s, 1H), 9.45 (s, 1H), 9 89 (broad, NH) ppm

3-Methylneoxanthobilirubic acid (7). To a solution of 5-formyl-4-methylpyrrole-3-propionic acid (**15**) (7 4 g, 40.6 mmole) and 3,4-dimethylpyrrolin-2-one (**7**) (5 4 g, 48 8 mmole) in 15 mL of methanol was added sodium hydroxide (21 g in 74 mL water) solution at room temperature The reaction mixture was stirred overnight (a precipitate formed after about 2 hours), and the alcohol was evaporated to leave a yellow aqueous solution This was diluted to 250 mL with water and acidified with 25 mL of acetic acid in an ice water bath to afford a fine yellow precipitate, which was filtered, washed with water and dried to give 10 1 g (90% yield) of fairly pure 3-nor-neo-XBR acid (**7**) It had mp 245-247°C, IR (KBr film) ν 3345, 3125, 2910, 1615 UV/vis: ϵ_{395}^{max} = 27,000 CH_3OH, ϵ_{395}^{max} = 30,000 $(CH_3)_2SO$); ^{1}H-NMR δ 1 96 (s, 3H), 2 21 (s, 3H), 2.23 (s, 3H), 2 70 (t, 2H, J=7 2 Hz), 2.80 (t, 2H, J=7 2 Hz), 6 65 (s, 1H), 7 02 (s, 1H), 9 54 (broad, NH), 11 98 (broad, NH) ppm ^{13}C-NMR δ: 7 82 (q), 9 56 (q), 10 11 (q), 20 13 (t), 34 44 (t), 109 54 (d), 121.40 (d), 123 81 (s), 124 25 (s), 125.36 (s), 127 83 (s), 129.45 (s), 145 82 (s), 172 22 (s), 179 98 (s) ppm The compound was analyzed (% C, H, N) as its methyl ester

Methyl 3-methylneoxanthobilirubinate. Excess etheral diazomethane was added to 3-methyl-neoXBR acid (**7**) (50 mg, 0 18 mmole) suspended in 10 mL of methanol The mixture was stirred for 1 hour after which

the solvent was allowed to evaporate. The residue was dissolved in 5 mL of dichloromethane and passed through a short column of silica gel (Woelm TLC grade F-DC 35/41, 18% water), eluting with dichloromethane. This procedure gave 44.6 mg (85% yield) of very pure methyl ester product. It had mp 195-197°C; IR (deposition from CH_2Cl_2) ν: 3325, 1675, 1645, 1455, 1265 cm^{-1}. UV/vis: ϵ_{388}^{max} = 29,500 ($CHCl_3$); ϵ_{394}^{max} = 27,500 (CH_3OH); ϵ_{394}^{max} = 31,000 ($(CH_3)_2SO$); ^{1}H-NMR δ: 1.94 (s, 3H), 2.12 (s, 3H), 2.14 (s, 3H), 2.58 (t, 2H, J=7.2 Hz), 2.78 (t, 2H, J=7.2 Hz), 3.68 (s, 3H), 6.12 (s, 1H), 6.82 (s, 1H), 10.32 (broad, NH), 10.68 (broad, NH) ppm, ^{1}H-NMR ($(CD_3)_2SO$) δ. 1.74 (s, 3H), 1.97 (s, 3H), 2.00 (s, 3H), 2.46 (t, 2H, J=7.2 Hz), 2.68 (t, 2H, J=7.2 Hz), 3.55 (s, 3H), 5.92 (s, 1H), 6.76 (s, 1H), 9.66 (s, 1H), 10.48 (s, 1H); ^{13}C-NMR δ: 8.18 (q), 9.31 (q), 9.84 (q), 20.72 (t), 34.81 (t), 51.52 (q), 101.02 (d), 120.52 (d), 122.54 (s), 123.25 (s), 124.16 (s), 124.36 (s), 129.72 (s), 142.39 (s), 173.70 (s), 174.15 (s) ppm. *Anal* Calcd for $C_{16}H_{20}N_2O_3$ (288) C, 66.67, H, 6.94, N, 9.72 Found C, 66.95; H, 6.97, N, 9.64.

10,10-Dimethyl-3,17-bis-nor-mesobilirubin-XIIIα (10,10-Dimethylglaucorubin) (1). To a mixture of 3 g (10.9 mmole) of 3-methyl-neo-XBR (7) and 1.1 g (10.6 mmole) of 2,2-dimethoxypropane was added cold (0°C) trifluoroacetic acid TFA (20 mL) with vigorous stirring. After 5 minutes, 500 mL of ice cold water was added to the reaction mixture. The yellow precipitate was collected by filtration, washed with water and dried by suction. The collected yellow precipitate was washed with dichloromethane (5 x 40 mL), and the yellow solution of dichloromethane was combined and saved. The residue which was not dissolved (starting pigment) weighed about 2.1 g. This was treated with 750 mg of 2,2-dimethoxypropane and 15 mL of cold TFA for 5 minutes, followed by the same treatment as above, which allowed for a rough separation of product and recovery of unreacted starting material. The cycle was repeated twice more. The yellow dichloromethane washings were combined and evaporated to dryness. The yellowish residue was dissolved in 200 mL of dichloromethane and allowed to pass a flash column (Woelm TLC grade F-DC 35/41 silica gel with 18% of water), eluting with dichloromethane, to afford 835 mg of pure 10,10-dimethylglaucorubin (1) after solvent evaporation and drying. The yield was 41%, based on recovery of 1.1 g of dark starting material. The new pigment (1) had mp 210°C (dec); IR (deposition from CH_2Cl_2) ν. 3445, 2978, 1705, 1694, 1677, 1640, 1615, 1590 cm^{-1}, ^{13}C-NMR and ^{1}H-NMR in Tables 1, 2 and 3 and UV-vis in Table 5. *Anal.* Calcd for $C_{33}H_{40}N_4O_6$ (588) C, 67.35, H, 6.80; N, 9.52. Found C, 67.13; H, 6.96, N, 9.31.

3,17-Bis-nor-mesobilirubin-XIIIα (Glaucorubin) (3). 3-Nor-neo-XBR (7) (1 g, 3.65 mmole) and 38% aq formaldehyde (4 mL) was treated with concentrated hydrochloric acid (3 mL) under vigorous stirring at room temperature for only 5 minutes, then 500 mL of ice cold water was added to quench the reaction. A greenish precipitate was collected by filtration and washed with methanol to give a crude yellowish product, which was recrystallized in from $CHCl_3$-CH_3OH to give 500 mg (49% yield) of fairly pure glaucorubin (3). It had mp 320-322°C; IR (deposition from CH_2Cl_2) ν 3420, 3260, 2965, 1703, 1685, 1630 cm^{-1}; ^{13}C-NMR and ^{1}H-NMR in Tables 1, 2 and 3 and UV-vis in Table 5. *Anal.* Calcd for $C_{31}H_{36}N_4O_6$ (560) C, 66.43, H, 6.43; N,10.00 Found. C, 66.04, H, 6.38, N, 10.07.

10,10-Dimethylglaucorubin dimethyl ester (5). 10,10-Dimethylglaucorubin (1) (100 mg, 0.170 mmole) was dissolved in 25 mL of dry $(CH_3)_2SO$. The mixture was heated at 40°C under a nitrogen atmosphere for 3 hours; then 20 mL of methanol was added, and the reaction was maintained under continuous stirring at the same temperature for 2 hours more. The mixture was poured into 100 mL water and extracted with dichloromethane (50 mL x 3). The combined organic phase was washed with 5% aq $NaHCO_3$, dried and evaporated to dryness. This gave pure dimethyl ester 3, 104 mg (99% yield). It had mp 218-220°C, IR (deposition from CH_2Cl_2) ν. 3345, 2928, 2855, 1736, 1655, 1643, 1634 cm^{-1}, ^{13}C-NMR and ^{1}H-NMR in Tables 1, 2 and 3 and UV-vis in Table 5. *Anal.* Calcd for $C_{35}H_{44}N_4O_6$ (616) C, 68.18; H, 7.14, N, 9.09 Found C, 68.17; H, 7.32, N, 9.12.

Glaucorubin dimethyl ester (6). By the same procedure described for the preparation of 5, 100 mg (0.178 mmole) of glaucorubin (3) was converted quantitatively into its dimethyl ester (4). It had mp 245-247°C, IR (deposition from CH_2Cl_2) ν. 3350, 2935, 1743, 1660, 1630 cm^{-1}, ^{13}C-NMR and ^{1}H-NMR in Tables 1, 2 and 3 and UV-vis in Table 5. *Anal.* Calcd for $C_{33}H_{40}N_4O_6$ (588) C, 67.35, H, 6.80, N, 9.50 Found C, 67.34, H, 7.08; N, 9.10.

2-Ethylxanthobilirubic acid (8). To a solution of 3.5 g (19 3 mmoles) of 5-formyl-4-methylpyrrole-3-propionic acid (**15**) and 3.4 g (25 mmoles) of crude 3,4-diethylpyrrolin-2-one (**10**)[18] in 8 mL of CH_3OH was added 10 g (178 mmoles) of KOH in 30 mL of H_2O at room temperature. The reaction was stirred overnight at room temperature. (A yellow precipitate was formed after about 1 hour of stirring) The alcohol in the reaction mixture was evaporated and 100 mL of H_2O was added to dilute the solution. Then 12 mL of acetic acid was used to acidify the basic reaction mixture, with cooling in an ice bath The resultant yellow precipitate was filtered, washed with water, and dried to give 5.1 g (88% yield) of **8** It had mp 234-235°C; UV-vis: ϵ_{394}^{max} = 27,500 (CH_3OH), ϵ_{395}^{max} = 29,500 ($(CH_3)_2SO$); IR (KBr film) ν 3345, 3125, 2910, 1615 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1 20 (m, 6H), 2.51 (m, 8H), 6 65 (s, 1H), 7 02 (s, 1H), 9.54 (broad, NH), 11 98 (broad, NH) ppm An analytical sample was obtained in the form of the corresponding methyl ester $C_{18}H_{24}N_2O_3$ following treatment of (**8**) with an excess amount of diazomethane, as described for the preparation of the methyl ester of (**7**) *Anal* Calcd for $C_{18}H_{24}N_2O_3$ (316): C, 68 32, H, 7 65, N, 8.86 Found: C, 68 07; H, 7.43, N, 8 70.

Methyl 2-Ethylxanthobilirubinate. Reaction with diazomethane, as with **7**, gave the desired methyl ester It had mp 187-190°C; IR (deposition from CH_2Cl_2) ν: 3328, 1675, 1645 cm^{-1}, ^{1}H-NMR δ· 1.19 (m, 6H), 2 15 (s, 3H), 2 41 (q, 4H), 2 58 (m, 8H), 2 79 (q, 4H), 3.68 (s, 3H), 6 15 (s, 1H), 6.81 (s, 1H), 10 46 (broad, NH), 11 05 (broad, NH) ppm *Anal* Calcd for $C_{18}H_{24}N_2O_3$ (316)· C, 68 32; H, 7 65, N, 8.86 Found. C, 68.07, H, 7.43; N, 8 70

10,10-Dimethyl-2,18-diethylmesobilirubin-XIIIα (2). To a mixture of 2-ethyl XBR (**8**) (250 mg, 0 8 mmole) and 2,2-dimethoxypropane (85 mg, 1 equiv) was added cold (0°C) trifluoroacetic acid (1 5 mL) under vigorous stirring After 5 min of stirring, 50 mL of ice cold water was added to quench the reaction The resulting yellow precipitate was collected, dried and allowed to pass a flash column of silica gel (Woelm TLC grade F-DC 35/41, 18% water) with the elution of CH_2Cl_2 to give 21 mg (8% yield) of a non-polar pigment, 10,10-dimethyl-2,18-diethylmesobilirubin-XIIIα (**2**). It had mp 210°C (dec); IR (deposition from CH_2Cl_2) ν 3445, 2975, 1705, 1695, 1677, 1640, 1615, 1585 cm^{-1}; UV-vis: ϵ_{434}^{max} = 51,000 ($CHCl_3$), ϵ_{426}^{max} = 42,500 (CH_3OH), ϵ_{425}^{max} = 57,500 ($(CH_3)_2SO$); ^{13}C-NMR in Table 1; ^{1}H-NMR in Tables 2 and 3 *Anal.* Calcd for $C_{37}H_{48}N_4O_6$ (664) C, 68.91, H, 7 51; N, 8 69 Found. C, 68 66, H, 7 53, N, 8 71.

2,18-Diethylmesobilirubin-XIIIα (4). Method A: Treatment of 250 mg (0 8 mmole) 2-ethyl XBR (**8**) and 0 5 mL of 38% aq formaldehyde with 1 mL of concentrated hydrochloric acid under vigorous stirring for 5 min at room temperature, followed by quenching with ice water gave a greenish precipitate. The precipitate was collected by filtration and washed with cold methanol The resulting dark yellow solid residue was dissolved in CH_2Cl_2 and flash chromatographed through a column of silica gel (TLC grade, 18% water), eluting with CH_2Cl_2 to afford 39 mg (16% yield) of pure 2,18-diethylmesobilirubin-XIIIα (**2**).

Method B 2-Ethyl XBR (**8**) (250 mg, 0 8 mmole), dimethoxymethane (300 mg, 3 2 mmoles) and *p*-toluenesulfonic acid monohydrate (100 mg) were dissolved in 5 mL dichloromethane and stirred for 12 hours at room temperature. The resulting yellowish solution was passed by flash chromatography through a column of silica gel (TLC grade, 18% water) eluting with CH_2Cl_2 to give 72 mg (30% yield) of pure 2,18-diethylmesobilirubin-XIIIα (**2**) (yield 30%) It had mp > 300°C (dec), IR (deposition from CH_2Cl_2) ν 3420, 3260, 2965, 1703, 1685, 1630 cm^{-1}, UV-vis ϵ_{431}^{max} = 58,500 ($CHCl_3$), ϵ_{427}^{max} = 58,000 ($(CH_3)_2SO$), ^{13}C-NMR in Table 1; ^{1}H-NMR in Tables 2 and 3 *Anal* Calcd for $C_{35}H_{44}N_4O_6$ (616): C, 68 18, H, 7 14; N, 9 09 Found C, 68 12, H, 7 22, N, 8 69

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