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A Water-Soluble Synthetic Bilirubin with Carboxyl Groups Replaced by Sulfonyl Moieties

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Summary. The first symmetrical bilirubin analog with CO_2H groups replaced by SO_3H, 8,12-*bis*-(2-sulfo-ethyl)-3,17-diethyl-2,7,13,18-tetramethyl-(10*H*,21*H*,23*H*,24*H*)-bilin-1,19-dione, was synthesized from methyl (2,4-dimethyl-5-ethoxycarbonyl-1*H*-pyrrol-3-yl) acetate in nine steps *via* the sulfonic acid analog of xanthobilirubic acid (*XBR*) and isolated as its disodium salt. The sulfonic acid group was introduced at an early stage of the synthesis by reaction of an intermediate, ethyl 4-(2-bromoethyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylate, with sodium sulfite. The disodium bilirubin disulfonate exhibits NMR spectroscopic properties rather similar to those of the parent carboxylic acid, mesobilirubin-XIIIα; however, its UV/Vis spectra are blue-shifted and broadened relative to those of the parent compound. Like mesobilirubin, the disulfonate displays a positive exciton chirality circular dichroism spectrum, albeit with weaker *Cotton* effects, in a buffered aqueous solution ($pH = 7.4$) containing a 2:1 molar ratio of human serum albumin.

Keywords. Pyrrole; Sulfonic acid; Spectroscopy; CD.

Introduction

Bilirubin-IXα (Fig. 1A), the yellow pigment of jaundice, is formed from heme during normal metabolism in humans and other mammals [1, 2] and owes its water insolubility and other properties to a persistent tendency to tuck its polar carboxylic acid and amide groups inward, linking them by hydrogen bonds [3, 4]. The most stable conformation, shaped like a ridge-tile and secured by six intramolecular hydrogen bonds (Fig. 1B), has been found in crystals of bilirubin [5] and its dicarboxylate dianion salt [6] as well as in solution [7, 8]. The ridge-tile conformation is believed to be important in the transport and metabolism of bilirubin [2, 9]. Analogs with vinyl groups reduced to ethyl (as in mesobilirubin-XIIIα), with alkyl substituents on the propionic acids [10], or even with electronegative substituents such as OCH_3 [11] or F [12] on the propionic acid chains all apparently retain the conformation-determining intramolecular hydrogen bonding motif, which determines the pigment's shape and properties. However, analogs with propionic acid groups transposed from ring carbons 8 and 12 to other sites on the pigment backbone (*e.g.* to 7 and 13 in mesobilirubin-IVα [13]) are much more polar. They cannot engage in intramolecular hydrogen bonding and behave completely differently in solution [13].

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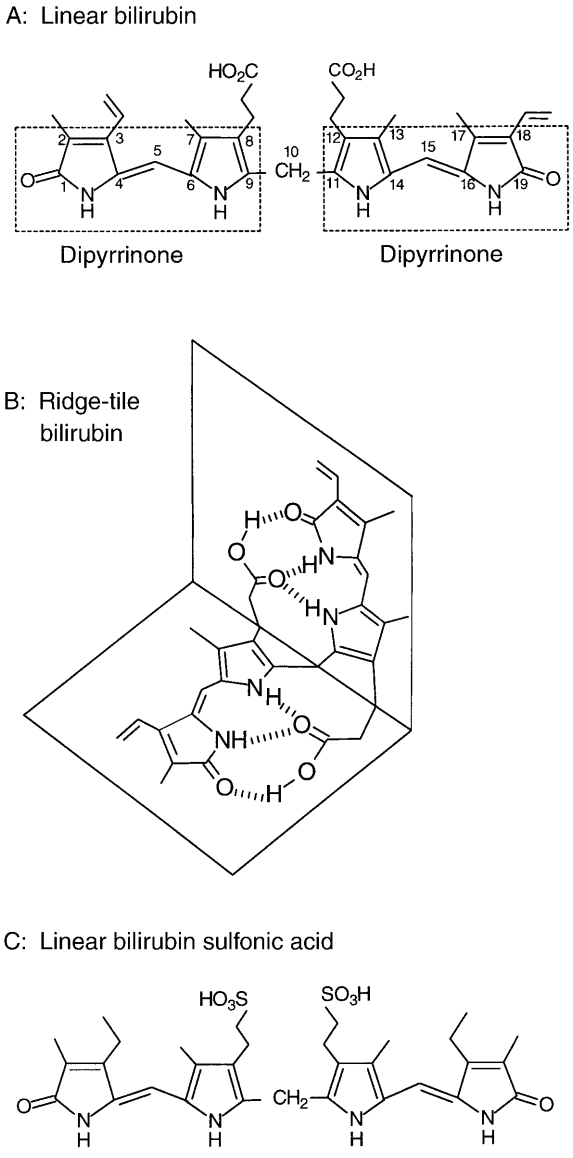


Fig. 1. Bilirubin-IXα shown in a linear representation (A) and in its most stable ridge-tile conformation (B); the ridge-tile is stabilized by a network of 6 intramolecular hydrogen bonds, only one of two enantiomers is shown; mesobilirubin-XIIIα with both CO_2H groups replaced by SO_3H groups (C)

The key elements required for intramolecular hydrogen bonding are thus a dipyrrinone and a partner carboxylic acid group, which must be tethered to ring carbons 8 and 12 (Fig. 1A, 1B). Carboxylic acid and dipyrrinone moieties form a strongly attractive complementary hydrogen bonding pair [14]. Even hydrogen bonding of the dipyrrinone to a carboxylate ion seems to be quite effective in retaining the pigment's folded, hydrogen-bonded conformation [6–8]. Although the carboxylic acid group appears to be a perfect match for a dipyrrinone receptor (and in bilirubin, the tight intramolecular hydrogen bonding between them renders the

pigment hydrophobic) when electronegative atoms such as F, or groups such as CH_3O are located adjacent to the CO_2H group, the profound increase in carboxylic acid acidity renders the pigment more polar and even water-soluble [11, 12]. In the following, we have extended our studies of acidity, hydrogen bonding, and solubility to a new bilirubin analog with CO_2H groups replaced by SO_3H (Fig. 1C).

Results and Discussion

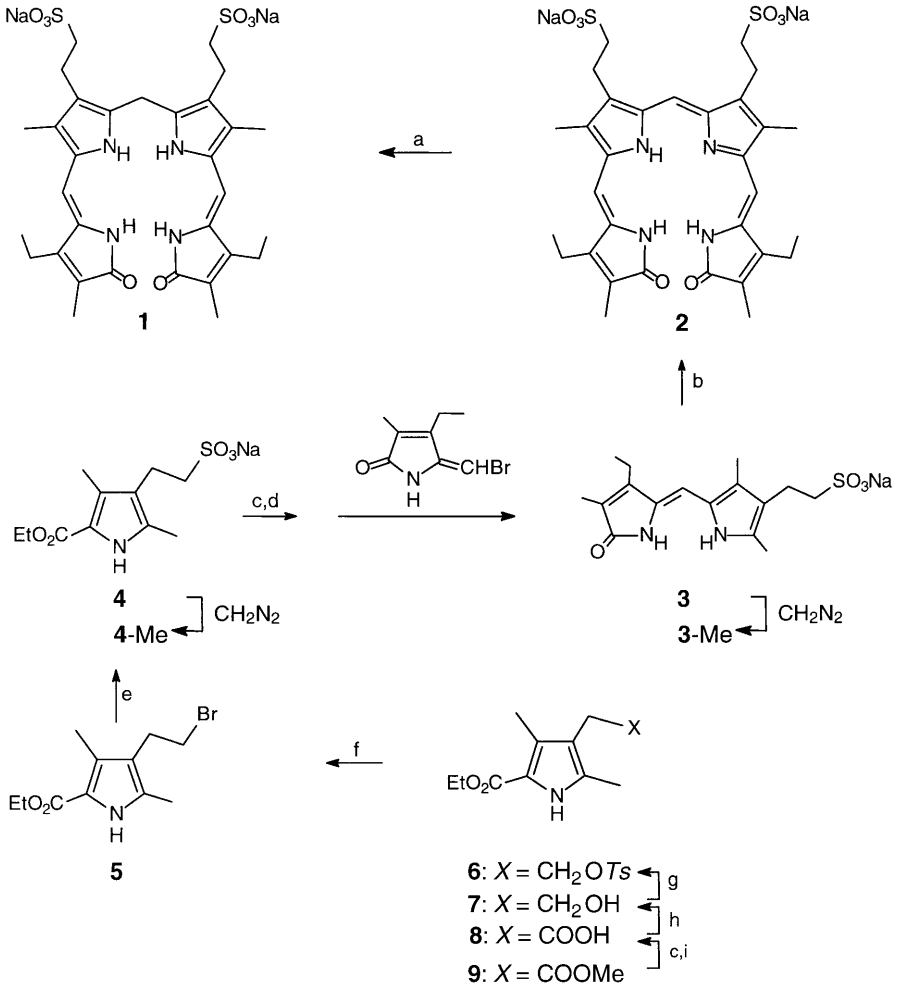
Synthesis aspects

In view of its ease of synthesis in high yield, we prepared monopyrrole **9** (Scheme I) as starting material for the eventual introduction of the sulfonic acid group in monopyrrole **4**. Introduction of the SO_3H group at a late stage of the synthesis seemed unattractive because the potential rubin intermediates are much less robust than their α-carboethoxypyrrole counterparts. Our approach was thus designed to introduce the SO_3H group early by displacing Br^- from the β-(2-bromoethyl)-pyrrole **5** with sodium sulfite. Synthesis of **5** was accomplished in a straightforward way by selectively saponifying **9** in aqueous-ethanolic NaOH to give **8** in 97% yield. Reduction of the free CO_2H group of **8** was achieved smoothly, selectively, and in high yield (96%) by treatment with BH_3-*THF* at $-30°C$. Tosylation of the resulting β-(2-hydroxyethyl)-pyrrole (**7**) afforded **6** in 82% yield, and **6** was converted to its β-(2-bromoethyl) derivative (**5**) by reaction with NaBr in *DMSO*. Reaction of **5** with Na_2SO_3 in aqueous dioxane led to a 79% yield of sulfonate **4**. Attempts to isolate the sulfonic acid parent of **4** proved to be rather difficult, but **4** could be converted to its methyl ester (**4**-Me), which was also isolated and characterized, by reaction with CH_2N_2.

Conversion of **4** to dipyrrinone **3** was achieved in a conventional [15] way: **4** was saponified to the corresponding carboxylic acid, which was reacted with 5-bromomethylene-4-ethyl-3-methyl-3-pyrrolin-2-one [16] to give a 59% yield of **3**, isolated as its sodium salt. As above, treatment of **3** with CH_2N_2 afforded a low yield of the methyl ester **3**-Me. *p*-Chloranil-promoted oxidative self-coupling [17] of **3** led to biliverdin **2**, and the isolated verdin disodium salt was reduced to rubin **1** by sodium borohydride. The isolated yields of salts **1** and **2** were low because it was difficult to remove inorganic salts.

Properties

As a measure of the relative polarity of sulfonic acids **1**–**3** and their carboxylic acid parents, we compared their behavior in reverse phase HPLC using 0.1 *M* di-*n*-octylamine acetate as eluent [18]. In this system, we observed that xanthobilirubic acid (*XBR*), mesobilirubin-XIIIα, and mesobiliverbin-XIIIα gave the same retention times as their corresponding sodium salts. We observed retention times of 6.56 min for **1** and 14.15 min for mesobilirubin-XIIIα; 4.77 min for **2** and 6.26 min for mesobiliverdin-XIIIα; and 3.67 min for **3** and 4.13 min for *XBR*. The long retention time of mesobilirubin-XIIIα relative to its verdin and *XBR* is due to its much greater lipophilicity and intramolecular hydrogen bonding. The shorter retention times of the ionized sulfonic acids relative to the ionized carboxylic acids are consistent with the former being more polar than the latter, and the longer retention time of **1**



a: $NaBH_4$/MeOH; b: *p*-chloranil; c: NaOH/H_2O/EtOH; d: HNO_3; e: Na_2SO_3; f: NaBr; g: *p*-*Ts*Cl/Et_3N; h: BH_3-*THF*; i: HCL

Scheme 1

relative to **2** and **3** is qualitatively consistent with **1** being less polar than either **2** or **3**, probably due to intramolecular hydrogen bonding in **1**.

The ^{13}C and ^{1}H NMR spectra of dipyrinnone **3**, verdin **2**, and rubin **1** compare favorably with their carboxylic acid analogs (Table 1). The chemical shifts are very similar and differ significantly only at carbons (and hydrogens) in the propionic acid chains. The presence of the $-SO_3Na$ group causes the α-carbons to become very strongly deshielded (by $\sim$17 ppm) relative to the CO_2H analog, but the β-carbons and hydrogens are not affected much. A similar behavior was noted with α,α'-difluoromesobilirubin-XIIIα [12]. Other than this noticeable difference, the chemical shifts of the remaining carbons and hydrogens match well with their carboxylic acid analogs.

The UV/Vis spectroscopic data (Table 2) of **2** and **3** differ only slightly from those of their parent carboxylic acid analogs in polar solvents: *DMSO* (Fig. 2A, B),

Table 1. Comparison of ^{13}C NMR assignments (5×10^{-3} to 1×10^{-2} *M*) in *DMSO*-d_6 at 25°C; δ/ppm of the sodium salts of the *bis*-sulfonic acid rubin (**1**) and verdin (**2**) and the sulfonic acid dipyrrinone (**3**) with their carboxylic acid analogs mesobilirubin-XIIIα (*MBR*), mesobiliverdin-XIIIα (*MBV*), and xanthobilirubic acid (*XBR*)

Position	**1**	*MBR*	**2**	*MBV*	**3**	*XBR*
1,19-CONH	171.74	171.93	172.29	172.28	171.84	171.89
2,18	122.38	122.51	127.58	127.66	122.28	122.29
2,18-CH_3	9.40	9.14	9.15	9.18	9.20	9.21
3,17	147.09	147.18	146.31	146.32	147.15	147.19
3,17-CH_2CH_3	17.18	17.15	17.00	16.99	17.14	17.15
3,17-CH_2CH_3	14.83	14.81	14.45	14.46	14.83	14.84
4,16	127.16	127.81	139.84	139.96	127.11	127.25
5,15-CH=	98.00	97.69	95.90	95.78	97.70	97.62
6,14	121.79	121.95	149.46	149.35	121.60	121.67
7,13	119.21	119.24	127.20	127.41	118.96	118.74
7,13-CH_3	8.07	8.07	8.13	8.15	8.05	8.06
8,12	122.44	122.91	138.12	137.95	122.52	122.63
β,β'-CH_2	19.88	19.25	20.37	19.24	20.10	19.45
α,α'-CH_2	51.38	34.34	52.43	35.24	52.31	34.95
α,α'-COOH	–	173.97	–	173.62	–	174.04
9,11	131.70	130.32	139.98	140.47	129.13	129.43
10	21.96	23.46	114.83	116.02	10.91	10.97

Table 2. Comparison of UV/Vis data (2×10^{-5} to 4×10^{-5} *M* in the given solvent containing 2% (v/v) *DMSO*; λ_{max}/nm (ε_{max}/mol^{-1} · dm^3 · cm^{-1})) of the sodium salts of the *bis*-sulfonic acid rubin (**1**) and verdin (**2**) and the sulfonic acid dipyrrinone (**3**) with their carboxylic acid analogs mesobilirubin-XIIIα (*MBR*), mesobiliverdin-XIIIα (*MBV*), and xanthobilirubic acid (*XBR*)

Solvent	**1**	*MBR*	**2**	*MBV*	**3**	*XBR*
DMSO	426 (50800) sh 401 sh (46800)	439 (59000) sh 410 sh (47700)	636 (9200) 374 (30400)	638 (11000) 372 (35800)	411 (29700)	411 (34400)
MeOH	426 (53100) sh 397 sh (45700)	438 (58200) sh 416 sh (48900)	639 (7900) 366 (29800)	641 (9400) 366 (35500)	411 (30800)	413 (36200)
MeCN	420 (50400) sh 398 sh (47800)	438 (56800)	624 (7800) 362 (28500)	641 (9700) 365 (36400)	403 (29700)	402 (34100)
Phosphate buffer ($pH = 8.60$)	421 (43600) sh 391 sh (39300)	432 (53400) sh 415 sh (50200)	657 (6900) 364 (27100)	656 (8600) 366 (33900)	406 (26800)	411 (31800)

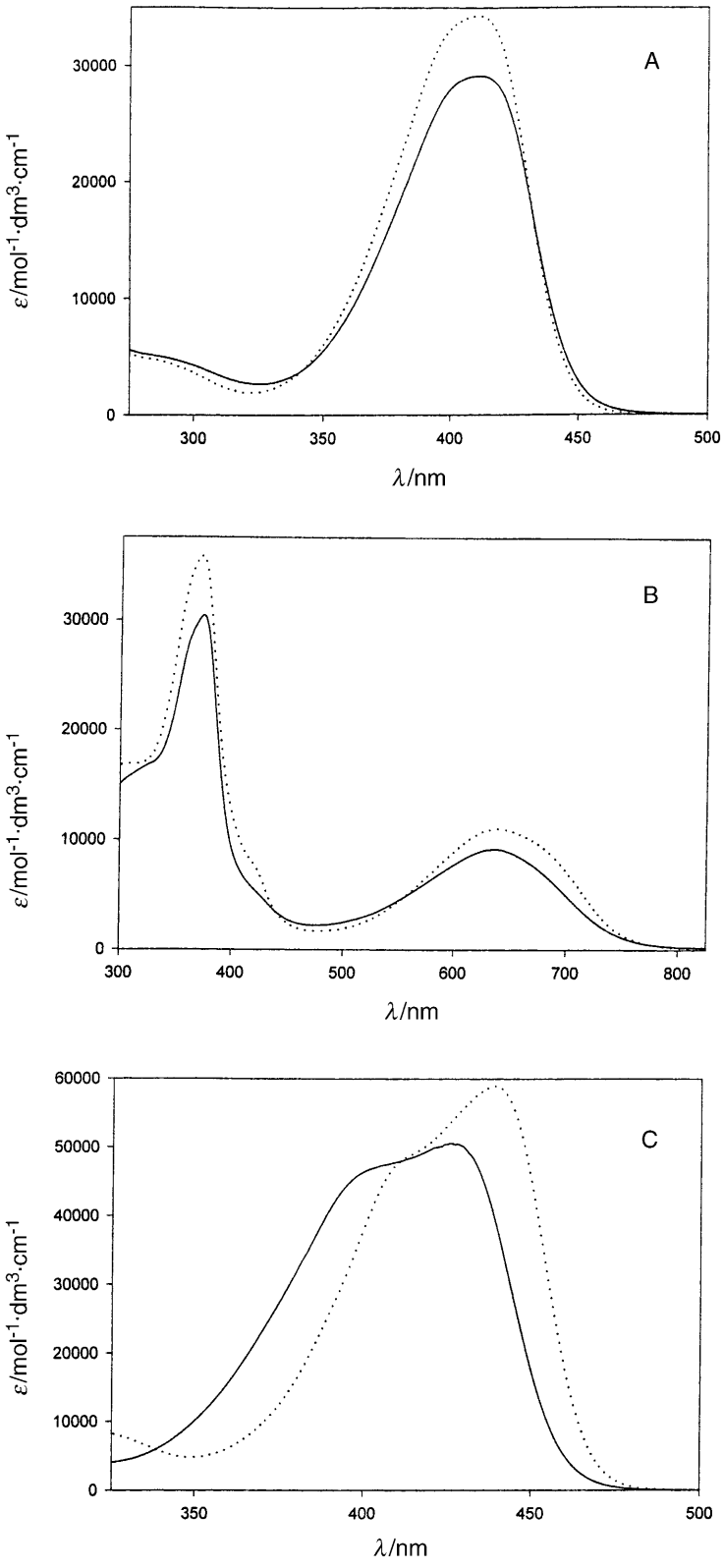


Fig. 2. UV/Vis spectra of **3** (A), **2** (B), and **1** (C) in *DMSO* (solid lines) as compared with their corresponding carboxylic acid analogs (dotted lines), xanthobilirubic acid, mesobiliverdin-XIIIα, and mesobilirubin-XIIIα, respectively; concentrations: *ca.* 10^{-5} M

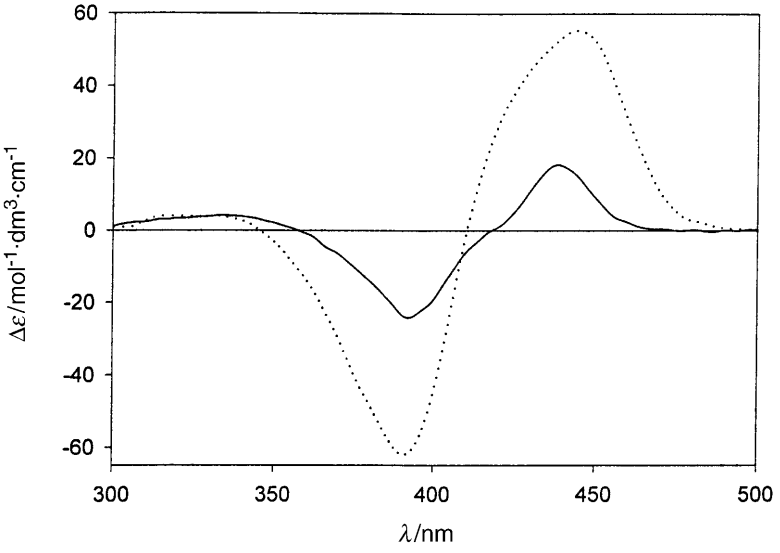


Fig. 3. Circular dichroism (CD) spectra of 4×10^{-5} *M* solutions of **1** (———) and mesobilirubin-XIIIα (· · · · · ·) in *pH* 7.4 aqueous phosphate buffer containing human serum albumin at 22°C; the molar ratio of pigment to protein is 1:2; CD data for **1**: $\Delta\varepsilon^{439}_{max} = +18, \Delta\varepsilon^{392}_{max} = -24$; CD data for mesobilirubin-XIIIα $\Delta\varepsilon^{444}_{max} = +55, \Delta\varepsilon^{391}_{max} = -62$

MeOH, H_2O, and MeCN. The slightly lower intensity of the long wavelength absorption (ε_{max}) of the sulfonic acid salts than that of the parent carboxylic acid may be attributed to the presence of inorganic salts in the former. Unlike **2** and **3**, in polar solvents the UV/Vis spectra of **1** (Fig. 2C) are hypsochromically shifted (by 11–12 nm) and slightly broadened. Both **1** and its parent, mesobilirubin-XIIIα, are molecular excitons [3], and their UV/Vis spectra clearly indicate (more clearly in **1** than in mesobilirubin-XIIIα) a splitting of the long-wavelength band. Their spectra are consistent with a ridge-tile conformation similar to that observed in carboxylate salts of bilirubin and mesobilirubin [6, 7].

Interestingly, solutions of **1** in *pH* 7.4 phosphate buffered human serum albumin (HSA) prepared as described previously [19a] for bilirubin-HSA solutions give bisignate induced circular dichroism *Cotton* effects of the exciton coupling type (Fig. 3) [3] as has been observed previously for mesobilirubin-XIIIα [19b]. The signed order of the *Cotton* effects remains the same in both **1** and mesobilirubin-XIIIα, indicating a preference for binding the same conformational enantiomer (Fig. 1) in the primary binding site of HSA [3, 19]. With **1**, the *Cotton* effect intensities are only about one-third of the magnitude of those of mesobilirubin-XIIIα, suggesting either poorer enantio-selectivity by the HSA or a weaker association complex, possibly due to the fact that **1** is water soluble, whereas mesobilirubin-XIIIα is highly insoluble. α,α'-Difluoromesobilirubin-XIIIα, which is also water-soluble, gave a qualitatively similar result from its CD in the presence of HSA [12].

Experimental

Nuclear magnetic resonance spectra (NMR) were recorded on GE GN-300 or Varian Unity Plus spectrometers operating at a proton frequency of 300 or 500 MHz. Chemical shifts are reported in δ

(ppm) and referenced to the residual $CHCl_3$ signal at 7.26 ppm (^1H) and $CDCl_3$ at 77.00 ppm (^{13}C). A *J*-modulated spin-echo experiment was used to obtain carbon multiplicities. The UV/Vis spectra were recorded on a Perkin Elmer Lambda 12 spectrophotometer. All solutions for UV/Vis measurements contained 2% (by volume) of *DMSO*. GC-MS analyses were carried out on a Hewlett-Packard 5890A capillary gas chromatograph (30 m DB-1 column) equipped with Hewlett-Packard 5970 mass selective detector. Analytical thin layer chromatography was carried out on J.T. Baker silica gel IB-F plates (Merck, 125 μm layer). Silica gel 70–230 mesh was employed for column chromatography. Melting points were determined on a Mel-Temp capillary apparatus and are uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ, and the data were within ±0.3% from the calculated values. High-resolution FAB mass spectra were obtained at the Nebraska Center for Mass Spectrometry, University of Nebraska, Lincoln, for samples which were >90% pure by NMR. Commercial reagents and HPLC grade solvents (Aldrich or Fischer) were dried and purified following standard procedures [20]. The starting ethyl 3,5-dimethyl-4-(methoxycarbonyl)-methyl-1*H*-pyrrole-2-carboxylate (**9**) was synthesized as previously described [21].

Ethyl 4-carboxymethyl-3,5-dimethyl-1H-pyrrole-2-carboxylate (**8**; $C_{11}H_{15}NO_4$)

A mixture of 11.96 g (50 mmol) of **9**, 2.20 g (55 mmol) of NaOH, 200 cm^3 of EtOH, and 25 cm^3 of H_2O was stirred for 24 h at room temperature. Then the ethanol was evaporated under vacuum, the residue was diluted with 350 cm^3 of H_2O, and carefully acidified by addition of 10% HCl at 0°C. The precipitated product was collected by filtration, washed with H_2O (2 × 100 cm^3), and dried under vacuum to afford monoacid **8**.

Yield: 10.97 g (97%); m.p.: 198–199°C (decomp.; Ref. [22]: 194–196°C); ^1H NMR ($CDCl_3$, δ): 1.34 (t, $J = 7.3$ Hz, 3H), 2.23 (s, 3H), 2.28 (s, 3H), 3.41 (s, 2H), 4.29 (q, $J = 7.3$ Hz, 2H), 9.12 (br s, 1H), 11.44 (br s, 1H) ppm; ^{13}C NMR ($CDCl_3$, δ): 10.63, 11.50, 14.52, 29.82, 59.93, 113.88, 117.22, 127.63, 131.25, 162.86, 177.11 ppm.

Ethyl 3,5-dimethyl-4-(hydroxyethyl)-1H-pyrrole-2-carboxylate (**7**; $C_{11}H_{17}NO_4$)

To a solution of 11.26 g (50 mmol) of **8** in 250 cm^3 of anhydrous *THF* cooled to −30°C under dry nitrogen, 65 cm^3 of 1 *M* BH_3-*THF* complex (65 mmol) were added during 1 h. After stirring for 2 h at −30°C, the mixture was allowed to warm slowly to room temperature over 22 h. The reaction was quenched by addition of 20 cm^3 *THF*: H_2O (1:1 by volume), and the *THF* was evaporated under vacuum. The residue was dissolved in 250 cm^3 of CH_2Cl_2 which was washed successively with 50 cm^3 of 0.2 *M* NaOH and H_2O (4 × 50 cm^3). The organic phase was dried over anhydrous $MgSO_4$ and filtered. The solvent was evaporated under vacuum, and the residue was recrystallized from ethyl acetate:hexane (*ca.* 1:3 by volume) to afford the pure hydroxy derivative **7**.

Yield: 10.18 g (96%); m.p.: 122–123°C (Ref. [22]: 121–123°C); MS *m/z* (rel. int.) = 211 ($M^{+\cdot}$, 14%), 180 (42%), 166 (8%), 134 (100%); ^1H NMR ($CDCl_3$, δ): 1.35 (t, $J = 7.2$ Hz, 3H), 2.23 (s, 3H), 2.28 (s, 3H), 2.65 (t, $J = 6.3$ Hz, 2H), 3.66 (t, $J = 6.3$ Hz, 2H), 4.30 (q, $J = 7.2$ Hz, 2H), 8.65 (br s, 1H) ppm; ^{13}C NMR ($CDCl_3$, δ): 10.58, 11.34, 14.44, 27.48, 59.63, 62.57, 117.03, 117.38, 127.31, 130.95, 161.94 ppm.

Ethyl 3,5-dimethyl-4-(2-p-toluenesulfonyloxyethyl)-1H-pyrrole-2-carboxylate (**6**; $C_{18}H_{23}NO_5S$)

To a solution of 6.34 g (30 mmol) of **7** in 75 cm^3 of anhydrous CH_2Cl_2 and 6.07 g (8.4 cm^3, 60 mmol) of triethylamine cooled to −5°C, 8.58 g (45 mmol) of *p*-toluenesulfonyl chloride were added during 1 h. The mixture was stirred for 2 h at 0°C and slowly warmed to room temperature over 14 h. Then the mixture was diluted with 100 cm^3 of CH_2Cl_2, washed with 2% HCl (50 cm^3) and water (4 × 50 cm^3), dried (anhydrous $MgSO_4$), and filtered. The solvent was evaporated under vacuum, and the residue recrystallized from ethyl acetate:hexane (*ca.* 1:2 by volume) to afford pure tosylate **6**.

Yield: 8.95 g (82%); m.p. 129–130°C (Ref. [22]: 126–128°C); ^{1}H NMR ($CDCl_3$, δ): 1.35 (t, $J = 7.2$ Hz, 3H), 2.11 (s, 3H), 2.14 (s, 3H), 2.42 (s, 3H), 2.71 (t, $J = 7.1$ Hz, 2H), 3.99 (t, $J = 7.1$ Hz, 2H), 4.29 (q, $J = 7.2$ Hz, 2H), 7.27 (d, $J = 8.0$ Hz, 2H), 7.67 (d, $J = 8.0$ Hz, 2H), 8.67 (br s, 1H) ppm; ^{13}C NMR ($CDCl_3$, δ): 10.23, 11.01, 14.36, 21.31, 23.83, 59.56, 69.73, 115.33, 116.90, 126.77, 127.45, 129.47, 131.06, 132.75, 144.40, 161.74 ppm.

Ethyl 4-(2-bromoethyl)-3,5-dimethyl-1H-pyrrole-2-carboxylate (**5**; $C_{11}H_{16}BrNO_2$)

A mixture of 3.65 g (10 mmol) of **6**, 60 cm^3 of anhydrous *DMSO*, and 10.29 g (100 mmol) of NaBr was heated briefly to 60°C to achieve dissolution of all tosylate and then stirred for 16 h at room temperature. Ice cold H_2O ($\sim$250 cm^3) was slowly added to precipitate the product which was filtered, washed with H_2O, and dried. Recrystallization from ethyl acetate:hexane (*ca*. 1:2 by volume) afforded pure bromide **5**.

Yield: 2.53 g (92%); m.p.: 145–146°C; MS: *m/z* (rel. int.) = 275, 273 ($M^{+\cdot}$, 32%), 230, 228 (11%), 194 (23%), 180 (80%), 148 (25%), 134 (100%); ^{1}H NMR ($CDCl_3$, δ): 1.35 (t, $J = 7.1$ Hz, 3H), 2.24 (s, 3H), 2.28 (s, 3H), 2.93 (t, $J = 7.8$ Hz, 2H), 3.36 (t, $J = 7.8$ Hz, 2H), 4.29 (q, $J = 7.1$ Hz, 2H), 8.78 (br s, 1H) ppm; ^{13}C NMR ($CDCl_3$, δ): 10.52, 11.59, 14.57, 28.22, 32.42, 59.76, 117.29, 118.98, 126.88, 130.16, 161.52 ppm.

Ethyl 3,5-dimethyl-4-(2-ethanesulfonic acid sodium salt)-1H-pyrrole-2-carboxylate (**4**; $C_{11}H_{16}NNaO_5S$)

To a solution of 2.74 g (10 mmol) of **5** in 30 cm^3 of purified 1,4-dioxane, a solution of 6.30 g (50 mmol) of Na_2SO_3 in 30 cm^3 of H_2O was added, and the mixture was heated at vigorous reflux for 9 h. After stirring for 16 h at room temperature, the supernatant was decanted and evaporated under vacuum to about 3 cm^3. Then, concentrated HCl was added until $pH < 7$. The precipitate was collected by filtration, washed with ice cold H_2O (2×5 cm^3), and dried under vacuum to afford sulfonate **4**.

Yield: 2.34 g (79%); m.p.: 284–286°C (decomp.); ^{1}H NMR (*DMSO*-d_6, δ): 1.24 (t, $J = 7.0$ Hz, 3H), 2.09 (s, 3H), 2.14 (s, 3H), 2.41 (m, 2H), 2.60 (m, 2H), 4.16 (q, $J = 7.0$ Hz, 2H), 11.04 (br s, 1H) ppm; ^{13}C NMR (*DMSO*-d_6, δ): 10.34, 10.76, 14.55, 19.85, 52.15, 58.66, 115.79, 119.57, 125.69, 130.24, 160.80 ppm; LRMS (FAB, 3-*NBA*) $m/z = 298.3$, 297.3.

Ethyl 3,5-dimethyl-4-(methyl 2-ethanesulfonate)-1H-pyrrole-2-carboxylate (**4**-Me; $C_{12}H_{19}NO_5S$)

A solution of 297 mg (1 mmol) of **4** in 15 cm^3 of MeOH was treated with excess of ethereal diazomethane until a yellow color persisted. Excess CH_2N_2 was destroyed by addition of 2–3 drops of acetic acid. Then the mixture was diluted with 50 cm^3 of $CHCl_3$, washed with H_2O (2×50 cm^3), and dried (anhydrous Na_2SO_4). After filtration and evaporation of the solvent under vacuum, the residue was purified by radial chromatography (eluent: 0.5–1.0% MeOH in CH_2Cl_2, v/v) to afford methyl sulfonate **4**-Me after recrystallization from ethyl acetate:hexane = 1:3.

Yield: 143 mg (49%); m.p.: 118–119°C; ^{1}H NMR ($CDCl_3$, δ): 1.35 (t, $J = 7.1$ Hz, 3H), 2.24 (s, 3H), 2.28 (s, 3H), 2.93 (*AB*A′B′, m, 2H), 3.15 (AB*A′B′*, m, 2H), 3.88 (s, 3H), 4.30 (q, $J = 7.1$ Hz, 2H), 8.68 (br s, 1H) ppm; ^{13}C NMR ($CDCl_3$, δ): 10.47, 11.27, 14.49, 18.40, 49.76, 55.03, 59.84, 116.91, 117.41, 126.42, 130.12, 161.63 ppm.

8-(2-Ethanesulfonic acid sodium salt)-3-ethyl-2,7,9-trimethyl-1,10-dihydro-11H-dipyrrin-1-one (**3**; $C_{16}H_{21}N_2NaO_4S$)

A mixture of 1.49 g (5 mmol) of **4**, 1.00 g (25 mmol) of NaOH, 15 cm^3 of EtOH, and 7.5 cm^3 of H_2O was heated at vigorous reflux for 3.5 h. The solvents were evaporated until dryness under vacuum. To

the residue, 1.08 g (5 mmol) of 5-bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole [16] and 17.5 cm^3 of anhydrous EtOH were added, and the mixture was acidified to $pH < 3$ with concentrated HNO_3. Then it was heated at reflux for 3 h and chilled overnight at −20°C. The precipitate was filtered, washed with 20 cm^3 of CH_2Cl_2, 2 × 10 cm^3 of cold H_2O, and dried in vacuum to afford dipyrrinone **3**.

Yield: 1.06 g (59%); m.p.: >320°C (decomp.); ^{1}H NMR (*DMSO*-d_6, δ): 1.06 (t, $J = 7.5$ Hz, 3H), 1.76 (s, 3H), 1.99 (s, 3H), 2.15 (s, 3H), 2.41 (m, 2H), 2.51 (q, $J = 7.5$ Hz, 2H), 2.58 (m, 2H), 5.91 (s, 1H), 9.74 (s, 1H), 10.26 (s, 1H) ppm; ^{13}C NMR (*DMSO*-d_6, δ): 8.05, 9.20, 10.91, 14.83, 17.14, 20.10, 52.31, 97.70, 118.96, 121.60, 122.28, 122.52, 127.11, 129.13, 147.15, 171.84 ppm; LRMS (FAB, 3-*NBA*): $m/z = 361.3, 360.3$; HRMS (FAB, 3-*NBA*): calcd.: 360.1120, found: 360.1114, $\Delta = 0.6$ mDa, error: 1.7 ppm; UV/Vis (*DMSO*): λ_{max} (ε) = 411 (29700) nm; UV/Vis (CH_3CN): λ_{max} (ε) = 403 (29700) nm; UV/Vis (H_2O): λ_{max} (ε) = 406 (27200) nm.

8-(Methyl 2-ethanesulfonate)-3-ethyl-2,7,9-trimethyl-1,10-dihydro-11H-dipyrrin-1-one (**3**-Me; $C_{17}H_{24}N_2O_4S$)

A solution of 180 mg (0.5 mmol) of **3** in 10 cm^3 of MeOH was treated with an excess of ethereal CH_2N_2 during 10 min. Then the excess CH_2N_2 was destroyed by addition of 3–4 drops of acetic acid, and the solution was diluted with 100 cm^3 of $CHCl_3$ and washed with H_2O (2 × 100 cm^3). The $CHCl_3$ layer was dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent, the residue was purified by radial chromatography eluting with 2–4% MeOH in CH_2Cl_2 (v/v) to yield the methyl sulfonate (**3**-Me) after recrystallization from MeOH:CH_2Cl_2 = 5:1.

Yield: 39 mg (22%); m.p.: 249–251°C; ^{1}H NMR ($CDCl_3$, δ): 1.18 (t, $J = 7.7$ Hz, 3H), 1.95 (s, 3H), 2.15 (s, 3H), 2.43 (s, 3H), 2.56 (q, $J = 7.7$ Hz, 2H), 2.97 (*AB*A′B′, m, 2H), 3.18 (AB*A′B′*, m, 2H), 3.89 (s, 3H), 6.12 (s, 1H), 10.41 (br s, 1H), 11.23 (br s, 1H) ppm; ^{13}C NMR ($CDCl_3$, δ): 8.53, 9.57, 11.51, 15.00, 17.95, 18.64, 50.02, 55.03, 100.71, 116.05, 122.72, 122.91, 123.97, 127.72, 131.59, 148.55, 174.25 ppm; ^{1}H NMR (*DMSO*-d_6, δ): 1.08 (t, $J = 7.6$ Hz, 3H), 1.77 (s, 3H), 2.04 (s, 3H), 2.19 (s, 3H), 2.50 (q, $J = 7.6$ Hz, 2H), 2.73 (*AB*A′B′, m, 2H), 3.33 (AB*A′B′*, m, 2H), 3.84 (s, 3H), 5.93 (s, 1H), 9.74 (s, 1H), 10.36 (s, 1H) ppm; ^{13}C NMR (*DMSO*-d_6, δ): 8.04, 9.09, 10.86, 14.81, 17.11, 18.06, 48.18, 56.40, 97.40, 115.70, 121.79, 122.11, 122.91, 127.65, 129.84, 147.21, 171.89 ppm.

8,12-Bis-(2-ethanesulfonic acid sodium salt)-3,17-diethyl-2,7,13,18-tetramethyl-(21H,24H)-bilin-1,19-dione (**2**; $C_{31}H_{36}N_4Na_2O_8S_2$)

A mixture of 720 mg (2 mmol) of **3**, 1.23 g (5 mmol) of *p*-chloranil, 500 cm^3 of CH_2Cl_2, and 24 cm^3 of formic acid was heated at reflux for 24 h. After cooling, MeOH (50 cm^3) was added, and the mixture was concentrated under vacuum to a volume of about 35 cm^3. This residue was chilled overnight at −20°C, and the precipitated yellow solid was separated by filtration and washed with cold MeOH to afford after evaporation of the bright blue filtrate crude mesobiliverdin-XIIIα-*bis*-sodium sulfonate which was used without further purification in the next step. A fraction (100 mg) of crude **2** from an independent synthesis was passed through a 6 cm column with silica gel (deactivated with 5% of water) and eluted with CH_2Cl_2:CH_3OH:NH_4OH = 100:7.5:0 to 100:25:5. Evaporation of the most polar bright blue fractions afforded a suitable sample for characterization by NMR.

Yield: 244 mg (35%); m.p.: >304°C (decomp.); ^{1}H NMR (*DMSO*-d_6, δ): 1.11 (t, $J = 7.4$ Hz, 6H), 1.69 (s, 6H), 2.03 (s, 6H), 2.51 (q, $J = 7.4$ Hz, 4H), 2.54 (m, 4H), 2.85 (m, 4H), 5.97 (s, 2H), 6.79 (s, 1H), 9.85 (br s, 2H), 11.3 (very br s, 1H) ppm; ^{13}C NMR (*DMSO*-d_6, δ): 8.13, 9.15, 14.45, 17.00, 20.37, 52.43, 95.90, 114.83, 127.20, 127.58, 138.12, 139.84, 139.98, 146.31, 149.46, 172.29 ppm; HRMS (FAB, 3-*NBA*): calcd.: 703.1848 (MH^+), found: 703.1855, $\Delta = 0.7$ mDa, error: 1.0 ppm.

8,12-Bis-(2-ethanesulfonic acid sodium salt)-3,17-diethyl-2,7,13,18-tetramethyl-(10H,21H,23H,24H)-bilin-1,19-dione (**1**; $C_{31}H_{38}N_4Na_2O_8S_2$)

A mixture of 211 mg (0.3 mmol) of crude **2** and 80 cm^3 of anhydrous MeOH was sonicated for 20 min while purging it with N_2. $NaBH_4$ (1.02 g, 30 mmol) was added in small portions during 15 min at 0°C. The blue mixture was stirred under nitrogen for 1 h at 0°C and for 30 min at room temperature when the color changed to light green. H_2O (100 cm^3) was added, and the mixture was acidified with concentrated HCl to $pH < 2$. The yellow product was extracted with $CHCl_3$ (5×50 cm^3) leaving a blue aqueous phase. The solvent from the combined extracts was evaporated under vacuum to afford mesobilirubin sulfonate **1**. Passing the aqueous solution of this material through a 60 cm long (10 mm ID) column packed with 20 g of Dowex 50W-X8 sulfonic acid resin (Baker, 50–100 mesh) and eluting with water led to 50% recovery of an orange solid whose ^1H and ^{13}C NMR spectra in *DMSO*-d_6 were identical to those of the crude mesobilirubin **1**.

Yield: 21 mg (10%); m.p.: >290°C (decomp.); ^1H NMR (*DMSO*-d_6, δ): 1.07 (t, $J = 7.3$ Hz, 6H), 1.74 (s, 6H), 2.05 (s, 6H), 2.51 (q, $J = 7.3$ Hz, 4H), 2.66 (t, $J = 6.2$, 6.6 Hz, 4H), 2.73 (t, $J = 6.6$ 6.2 Hz, 4H), 3.94 (s, 2H), 5.92 (s, 2H), 9.84 (s, 2H), 10.54 (s, 2H) ppm; ^{13}C NMR (*DMSO*-d_6, δ): 8.07, 9.40, 14.83, 17.18, 19.88, 21.96, 51.38, 98.00, 119.21, 121.79, 122.38, 122.44, 127.16, 131.70, 147.09, 171.74 ppm; HRMS (FAB, 3-*NBA*): calcd.: 704.1926, found: 704.1906, $\Delta = 2.0$ mDa, error: 2.9 ppm.

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