# Synthesis of the First Fluorinated Bilirubin

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A symmetrical difluorinated bilirubin analog, 8,12-bis(2-carboxy-2-fluoroethyl)-3,17-diethyl-2,7,-13,18-tetramethyl-10*H*,21*H*,23*H*,24*H*-biline-1,19-dione (**9**), was synthesized from methyl 3-[2,4-dimethyl-5-(methoxycarbonyl)-1*H*-pyrrol-3-yl] propionate (**1**) in nine steps. Fluorine was introduced by reaction of an intermediate methyl 3-[1-(*tert*-butoxycarbonyl)-2,4-dimethyl-5-(methoxycarbonyl)-1*H*-pyrrol-3-yl]-2-hydroxypropionate (**5**), with (diethylamino)sulfur trifluoride (DAST). The fluorinated rubin exhibited the expected IR, UV−vis, and NMR spectroscopic properties, similar to those of the unfluorinated parent, mesobilirubin XIIIα. However, the solubility properties unexpectedly differed, with the fluorinated rubin being less soluble in organic solvents than its parent. While this phenomenon may be attributed to the much increased acidity of the carboxylic acid hydrogens in **9**, it probably also arises from less effective intramolecular hydrogen bonding due to a decreased basicity of the propionic acid carbonyl groups.

## Introduction

Bilirubin IXα, the yellow-orange pigment of jaundice formed from heme during normal metabolism in humans and other mammals,[1,2] owes its peculiar solubility and solution properties to a stubborn tendency to tuck its polar carboxylic acid and amide groups inward, linking them up through hydrogen bonding (Figure 1).[3,4] The most stable and persistent conformation, shaped like a ridge-tile and maintained by six intramolecular hydrogen bonds, has been found in crystals of bilirubin[5,6] as well as in various solutions[7,8] and is thought to be important in its transport and metabolism.[2,9] Analogs that have the C-8 and C-12 propionic acid groups transposed to other sites on the pigment backbone (e.g., C-7 and C-13 in mesobilirubin IVα) are much more polar, behave completely differently, and do not engage in intramolecular hydrogen bonding.[9,10] But analogs with propionic acids at C-8 and C-12 (as in mesobilirubin XIIIα) or replaced by a wide range of differing alkanoic acid chain lengths apparently do retain the conformation-determining intramolecular hydrogen bonding motif, which determines their shapes and properties.[9,11]

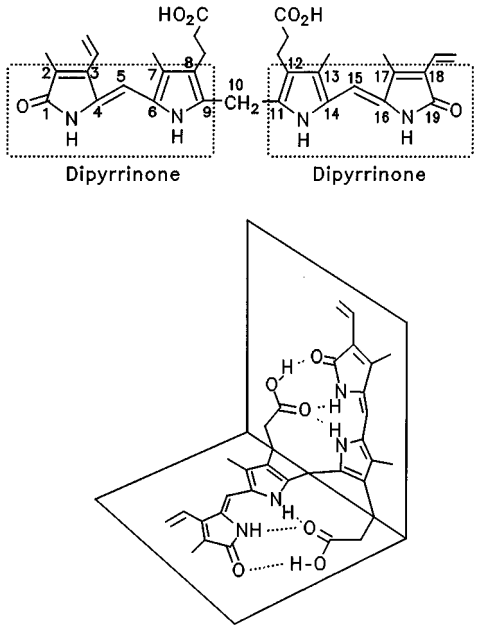


**Figure 1.** Bilirubin-IXα shown in a linear representation (upper) and in its most stable ridge-tile conformation (lower, only one of two enantiomers is shown). The ridge-tile is stabilized by a network of 6 intramolecular hydrogen bonds.

The key elements for intramolecular hydrogen bonding are thus a dipyrrinone receptor for the carboxylic acid group and a carboxylic acid tethered to ring carbons 8 and 12 (Figure 1). The carboxylic and dipyrrinone moieties form a complementary hydrogen bonding pair. Esterification disrupts the hydrogen bonding to some extent, as does amidation to give tertiary amides.[12] Even so, hydrogen bonding of the dipyrrinone to a carboxylate ion seems to be quite effective in retaining the pigment's folded, hydrogen-bonded conformation.[7,8] Since the carboxylic acid group is essential for effective hydrogen bonding to a dipyrrinone receptor, we wondered whether varying the acidity of the carboxylic acids might alter the effectiveness of the intramolecular hydrogen bonding. To achieve a profound alteration in carboxylic acid acidity, we determined that introduction of an α-fluorine, as in

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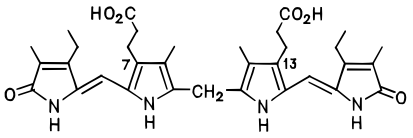
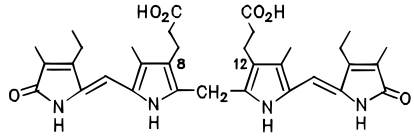
α,α′-difluoromesobilirubin XIIIα, should increase the propionic acid acidity constant by ~100-fold while avoiding the introduction of a large group. (The predicted increase in acidity due to fluorine substitution is based on the following determinations: $pK_a$ ($CH_3CO_2H$) = 4.76, $pK_a$ ($FCH_2CO_2H$) = 2.58.[13]).

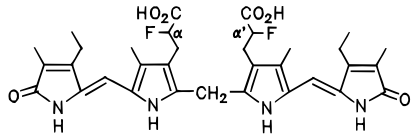
With its small van der Waals radius (1.35 Å) and short C−F bond length (1.39 Å), fluorine is thought to resemble hydrogen (van der Waals radius, 1.20 Å and C-H bond length, 1.43 Å).[14] It has been used as a "same steric size" replacement for hydrogen in pharmacophores to probe biological activity.[14] However, in view of its strong electronegativity, the fluorine substituent is often found to modify biological activity, as in a diverse array of pharmaceuticals such as 9α-fluorocortisone acetate, 5-fluorouracil, and fluorinated prostaglandins. It has been used as a mechanistic probe in biological transformations; e.g., high specificity of the *ω*-fluorine has been noted for pharmacological activity in *ω*-fluoro fatty acids; and α-(fluoromethyl) amino acids (potential irreversible inhibitors of amino acid decarboxylases); and 2-fluorourocanic acid has been found to be an irreversible inhibitor of urocanase. Although fluorinated porphyrins have been synthesized,[15] fluorinated bile pigments remain unknown.



Mesobilirubin IVα



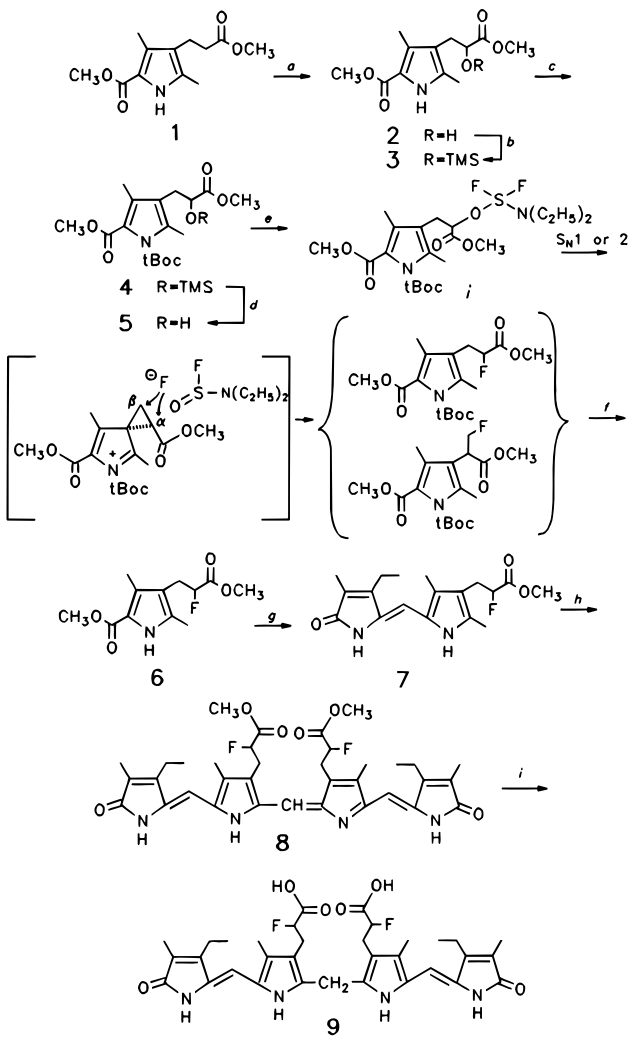
Mesobilirubin XIIIα



α,α′-Difluoromesobilirubin XIIIα

The natural bile pigment, bilirubin IXα, was first synthesized long ago,[16] and since that time many different analogs have been prepared.[17] One of the relatively easily synthesized bilirubin analogs is mesobilirubin XIIIα,[10,18] which adopts the same intramolecularly hydrogen-bonded ridge-tile conformation as bilirubin and exhibits quite similar solubility, solution, binding, metabolism and spectroscopic (*mutatis mutandis*) properties as bilirubin IXα. Introduction of one fluorine at each α-carbon of the two propionic side chains, as in α,α′-difluoromesobilirubin XIIIα, seemed feasible and attractive. The fluorine is not expected to alter the steric size of the propionic acid chain,[14] which is so important in maintaining tight intramolecular hydrogen bonding, but it should profoundly increase the carboxylic acid acidity.

**Scheme 1**[a]



[a] Key: (a) LDA, $O_2$, $P(OEt)_3$; (b) TMSBr, $Et_3N$; (c) $O[CO_2C(CH_3)_3]_2$, $Et_3N$, DMAP; (d) *n*-$Bu_4NF$; (e) DAST; (f) TFA, then fractional recrystallization; (g) $NaOH/H_2O$, then $HNO_3$, then 5-(bromomethylene)-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole; (h) *p*-chloranil, HCOOH; (i) $NaOH/H_2O$, then HCl, then $NaBH_4$, $CH_3OH$.

## Results and Discussion

**Synthesis.** In view of its ease of synthesis, in high yield, we focused our attention on monopyrrole **1** (Scheme 1) as starting material for the introduction of fluorine. Introduction of fluorine at the later stages of the synthesis were less attractive, in view of the greater sensitivity of the dipyrrinone unit relative to the α-carbomethoxypyrrole. Our approach was to introduce the fluorine at the propionic ester α-carbon following treatment by (diethylamino)sulfur trifluoride (DAST) of the

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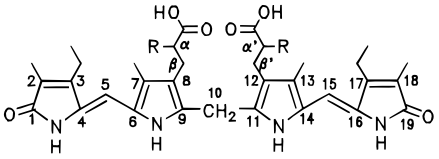
corresponding α-hydroxy ester. Synthesis of the latter was accomplished in a straightforward way by reacting **1** with LDA at −45 °C to form the α-anion and quenching with $O_2$. The α-hydroperoxide was reduced with triethyl phosphite to offer a 40% yield of pure **2**. However, the pyrrole ring of **2** was too reactive toward DAST, and so the NH was protected as the *t*-BOC derivative. First the α-OH was protected as its TMS ether (**3**), which was reacted with di-*tert*-butyl dicarbonate in triethylamine with added DMAP to afford the TMS-*t*-BOC derivative **4**. Then, the TMS ether of **4** was removed selectively using ($n$-Bu$N^+F^-$) to afford **5** in 73% yield from **2**. Reaction of **5** with DAST at −60 °C in $CH_2Cl_2$ gave 90% of a 1.2:1 ratio of desired and rearranged fluorinated *t*-BOC derivatives. The rearrangement occurring as a side reaction in the conversion of **5** → **6** (step e) appears to be similar to phenonium ion rearrangements,[19] for which analogous carbocation rearrangements in the β-pyrrolylethyl system have been reported by Smith *et al.*[20] Thus, reaction of **5** with DAST is expected to form initially the reactive intermediate ***i***. Neighboring group participation by the electron-rich pyrrole nucleus in the ionization of ***i*** leads to an ethylenepyrrolonium cation, paired to diethyl(fluorosulfoxy)amine and fluoride ion.[21] Attack by $F^-$ at either the α or β cyclopropyl carbon gives the desired α-fluoropropionic ester group and the rearranged α-(fluoromethyl)acetic ester group. Separation of the product mixture was achieved by crystallization following removal of the *t*-BOC group using TFA. This led to a 31% yield of the key monofluoropyrrole diester **6**. The presence of the fluorine was easily detected by $^{19}$F-NMR, and strong one-bond and two-bond $^{19}F-^{13}C$ couplings were found in the $^{13}$C-NMR of carbons in the propionic ester chain.

Diester **6** was saponified and then decarboxylated (pyrrole α-$CO_2H$) *in situ* while smoothly condensing the α-free pyrrole with 5-(bromomethylene)-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole to afford yellow dipyrrinone **7** in 74% yield after radial chromatography followed by crystallization. Oxidative coupling of **7** using *p*-chloranil afforded a 78% yield of the bright blue α,α′-difluoromesobiliverdin XIIIα dimethyl ester (**8**)—an unseparated mixture of racemic and meso diastereomers. In view of the ease of reduction of α-fluoro esters to β-fluoro alcohols using $NaBH_4$, we saponified **8** first and then reduced the verdin diacid to the rubin diacid with $NaBH_4$. This afforded a 39% yield of bright yellow α,α′-difluoromesobilirubin-XIIIα (**9**), as a mixture of *R*,*S*-diastereomers.

**Properties.** The $^{13}$C- and $^1$H-NMR spectra of **9** may be compared with its parent, mesobilirubin XIIIα (Table 1). The data are very similar, differing significantly only at carbons and hydrogen in the propionic acid chains. Thus, the α-carbons and hydrogens are very strongly deshielded, and the β-carbons and hydrogens are less strongly deshielded. Interestingly, while the COOH hydrogens are more deshielded in **9**, the COOH carbons are more shielded. As expected, the influence of the fluorine on the $^{13}$C chemical shifts falls off with distance, e.g., pyrrole ring carbons 8 and 12 are most strongly affected and ring carbons 7 and 13 or 9 and 11 much less strongly affected. The multiplicity of various carbon signals in **9** probably reflects the fact that **9** is a mixture of racemic and meso diastereomers.

**Table 1. Comparison of $^1$H- and $^{13}$C-NMR Spectral Data for α,α′-Difluoromesobilirubin XIIIα and Mesobilirubin XIIIα in $(CD_3)_2SO$ at 23 °C**



| | $^1$H-NMR signal | | $^{13}$C-NMR signal | |
|---|---|---|---|---|
| position | R = F | R = H | R = F | R = H |
| 1,19-CO | | | 172.01 | 171.9 |
| 2,18 | | | 123.18, 123.20[i] | 122.5 |
| 2,18-$CH_3$ | 1.77 | 1.78 | 9.41 | 9.14 |
| 3,17 | | | 147.25 | 147.2 |
| 3,17−$CH_2$$CH_3$ | 2.50[a] | 2.50[f] | 17.17 | 17.15 |
| 3,17-$CH_2$$CH_3$ | 1.08[b] | 1.09[g] | 14.87 | 14.81 |
| 4,16 | | | 128.18 | 127.8 |
| 5,15-CH= | 5.95 | 5.94 | 97.58 | 97.69 |
| 6,14 | | | 122.17, 122.21[i] | 122.0 |
| 7,13 | | | 123.30 | 122.9 |
| 7,13-$CH_3$ | 1.99 | 2.00 | 8.11 | 8.07 |
| 8,12 | | | 114.68, 114.71[i,j] | 119.2 |
| | | | 114.75, 114.77[i,j] | |
| β,β′-$CH_2$ | 2.67[c] | 2.43[h] | 26.78, 27.07[k] | 19.25 |
| α,α′-CH | 4.56[d] | 2.00[h] | 87.53, 89.94[l] | 34.34[n] |
| α,α′-COOH | 13.15 | 11.83 | 170.6, 170.9[m] | 174.0 |
| 9,11 | | | 131.21, 131.25[i] | 130.3 |
| 10-$CH_2$ | 3.98[e] | 3.95 | 23.58 | 23.46 |
| 21,24-NH | 9.80, 9.82 | 9.72 | | |
| 22,23-NH | 10.37, 10.40 | 10.27 | | |

[a] q, $J$ = 7.4 Hz. [b] t, $J$ = 7.4 Hz. [c] m. [d] 3 × m, $^2J_{FH}$ = 48.5 Hz. [e] s and AB. [f] q, $J$ = 7.6 Hz. [g] t, $J$ = 7.6 Hz. [h] $-CH_2-$, t, $J$ = 7.8 Hz. [i] Racemic and meso. [j] d, $^3J_{FC}$ = 2.2 Hz. [k] d, $^2J_{FC}$ = 21.9 Hz. [l] d, $^1J_{FC}$ = 182.1 Hz. [m] d, $^2J_{FC}$ = 23.8 Hz. [n] $-CH_2-$.

**Table 2. Comparison of UV−Vis Spectral Data for 2 × $10^{-5}$ M Solutions of 9 and its Parent, Mesobilirubin XIIIα**

| solvent | α,α′-$F_2$ (**9**) $\epsilon^{max}$ ($\lambda$, nm) | mesobilirubin XIIIα $\epsilon^{max}$ ($\lambda$, nm) |
|---|---|---|
| benzene | 48 000 (417) | 58 800 (435) |
| | | 54 700 (417)[sh] |
| $CHCl_3$ | 50 000 (420) | 57 800 (431) |
| THF | 47 500 (414) | 54 200 (428) |
| | 46 200 (394) | |
| $CH_3OH$ | 58 000 (422) | 50 700 (426) |
| | | 43 100 (401)[sh] |
| $(CH_3)_2SO$ | 55 700 (421) | 57 000 (426) |
| | 51 800 (395)[sh] | 49 100 (397)[sh] |

The UV−vis spectral data (Table 2) of **9** differ only slightly from those of the parent, mesobilirubin XIIIα. The intensity of the long wavelength absorption ($\epsilon^{max}$) is sometimes slightly lower than the parent, and $\lambda^{max}$ has undergone a slight hypsochromic shift. The data suggest a more helical conformation for **9** than in the parent or a ridge-tile (Figure 1) with a smaller interplanar angle.[4]

Interestingly, solutions of **9** in pH 7.4 aqueous buffered human serum albumin (HSA) prepared as described previously[22a] for bilirubin−HSA solutions, give bisignate, induced circular dichroism Cotton effects of the exciton coupling type (Figure 2)[4]—as has been observed previously for mesobilirubin XIIIα.[22b] The signed order of the Cotton eeffects remains the same in both **9** and its parent, indicating a preference for binding the same conformational enantiomer (Figure 1) to the HSA.[4,8,22] With **9**, the

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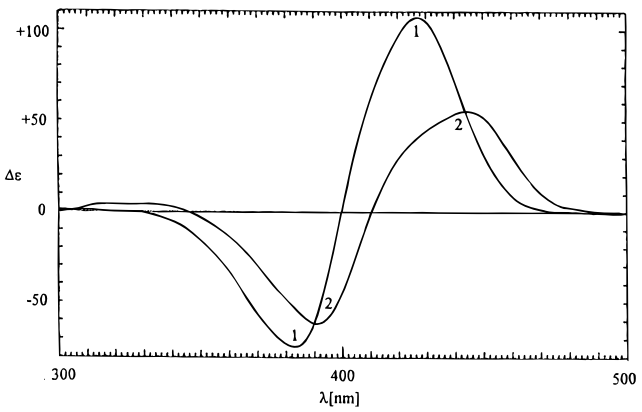


**Figure 2.** Circular dichroism (CD) spectra of $2 \times 10^{-5}$ M solutions of α,α′-difluoromesobilirubin-XIIIα (**9**) (Spectrum 1) and mesobilirubin-XIIIα (Spectrum 2) in pH 7.4 aqueous phosphate buffer containing human serum albumin in 22 °C. The molar ratio of pigment to protein is 1:2. CD and UV-vis data for **9**: $\Delta\epsilon_{427}^{max} = +107$, $\Delta\epsilon_{383}^{max} -74.5$, and $\epsilon_{429}^{max} = 50{,}200$; and for mesobilirubin-XIIIα: $\Delta\epsilon_{444}^{max}$ +55.3, $\Delta\epsilon_{391}^{max}$ −62.0, and $\epsilon_{437}^{max} = 46{,}600$.

Cotton effect intensities are larger, suggesting a greater enantioselectivity—possibly due to the more acidic carboxylic acids of **9** forming a tighter salt linkage to an amine residue (lysine) on HSA.[22]

If the spectral data for **9** generally conform to expectations, the solubility and chromatographic data were surprising. The parent mesobilirubin XIIIα is soluble in chloroform (~1 mg/mL maximum), but **9** is very insoluble—so much so that $^1$H-NMR could not be measured in this solvent. It also exhibits very limited solubility in most other organic solvents, although the solubility was sufficient for determining the UV−vis spectra, which were quite like that of mesobilirubin XIIIα. Dimethyl sulfoxide proved to be the best solvent for **9**, but it is not the solvent of choice for studying bilirubin conformation by NMR.[8] Comparison of TLC behavior on silica gel using 4% $CH_3OH$ in $CH_2Cl_2$ as irrigant gave an $R_f$ of 0.95 for mesobilirubin-XIIIα and $R_F$ values of 0.39 and 0.36 for **9** (diastereomeric mixture). The data suggest that the two fluorines of **9** render it more polar than the parent. Comparison of HPLC behavior using a reversed-phase column and 0.1 M di-*n*-octylamine acetate in methanol as eluent[23] gave a retention time of 16.5 and 17.1 min for **9** *vs* 17.9 min for mesobilirubin XIIIα. Consistent with **9** being a mixture of diastereomers, it exhibits two peaks (1:1 ratio). These data suggest that **9** is only somewhat more polar than its parent.

## Concluding Comments

The decreased solubility of α,α′-difluoromesobilirubin XIIIα (**9**) in organic solvents and its increased polarity, compared to the parent rubin, were unanticipated and suggest that intramolecular hydrogen bonding in **9** may be weaker or less influential. Other rubin acids that cannot hydrogen bond, e.g., mesobilirubin IVα, are also more polar and insoluble in solvents such as chloroform.[9] We surmise that the powerful inductive effect of the α-fluorine, in addition to rendering the carboxylic acid proton much more acidic than ordinary carboxylic acids,[13] also renders the nonbonded electrons of the carboxylic acid carbonyl much less basic (as reflected in the more shielded $^{13}$COOH chemical shift, Table 1)—and thus possibly less effective in maintaining the hydrogen-bonded structure of Figure 1. Metabolism studies of **9** and the influence of the enhanced carboxylic acid acidity on hepatic glucuronidation and excretion are in progress.

## Experimental Section

NMR spectra were obtained at 300 MHz and 500 MHz in $CDCl_3$ solvent (unless otherwise noted), and chemical shifts were reported in $\delta$ ppm. *J*-modulated spin-echo experiments (APT) were used to obtain $^{13}$C-NMR spectra. $^{19}$F-NMR spectra were referenced to external $CFCl_3$ standard at 0.00 ppm. GC−MS analyses were carried out on a capillary gas chromatograph (30 m DB-1 column) equipped with a mass selective detector. Analytical thin layer chromatography (TLC) was carried out on J. T. Baker silica gel IB-F plates (125 $\mu$m layer) using 4% methanol in dichloromethane. Radial chromatography was carried out on Merck silica gel $PF_{254}$ with $CaSO_4$ preparative layer grade. HPLC analyses were carried out on a high-performance liquid chromatograph with a UV−vis spectrophotometric detector (set at 410 nm) and a Beckman-Altex ultrasphere-IP 5 $\mu$m C-18 ODS column (25 × 0.46 cm) with Beckman ODS precolumn (4.5 × 0.46 cm). The flow rate was 1.0 mL/min, and the elution solvent was 0.1 *M* di-*n*-octylamine acetate in 5% aqueous methanol (pH 7.7, 34 °C). Melting points 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. Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). Ethyl acetoacetate, pentane-2,4-dione, methyl methacrylate, diisopropylamine, trimethylbromosilane, di-*tert*-butyl dicarbonate, tetra-*n*-butyl ammonium fluoride, *n*-butyllithium in hexane, (diethylamino)sulfur trifluoride (DAST), *p*-chloranil, and sodium borohydride were from Aldrich. Tetrahydrofuran, dichloromethane, chloroform, methanol, hexane, and dimethyl sulfoxide were HPLC grade from Fisher. Tetrahydrofuran was dried by distillation from $LiAlH_4$; methanol was distilled from $Mg(OCH_3)_2$; dimethyl sulfoxide was freshly distilled from $CaH_2$ under vacuum. Human serum albumin was defatted, from Sigma Chemical Co.

**Methyl 3-[2,4-Dimethyl-5-(methoxycarbonyl)-1*H*-pyrrol-3-yl]propionate (1).** This pyrrole was prepared as reported previously in 45% yield from methyl acetoacetate and methyl 4-acetyl-5-oxohexanoate: mp 106−107 °C (lit.[24] mp 107−108 °C); $^1$H-NMR $\delta$ 2.21 (3H, s), 2.26 (3H, s), 2.42 (2H, t, $J$ = 7.4, 8.3 Hz), 2.70 (2H, t, $J$ = 7.4, 8.3 Hz), 3.65 (3H, s), 3.81 (3H, s), 8.88 (1H, br s) ppm; $^{13}$C-NMR $\delta$ 10.41, 11.13, 19.45, 34.77, 50.75, 51.31, 116.6, 119.8, 126.8, 130.4, 162.3, 173.4 ppm; MS *m*/*z* (rel intens) 239 ($M^{\bullet+}$; 35), 208 (6), 180 (4), 166 (82), 134 (100), 106 (8) amu.

**Methyl 3-[2,4-Dimethyl-5-(methoxycarbonyl)-1*H*-pyrrol-3-yl]-2-hydroxypropionate (2).**[25] To a solution of LDA (prepared from 50 mmol of diisopropylamine and 50 mmol of 1.6 M *n*-BuLi in hexane at −20 °C) in 60 mL of dry THF was added a solution of **1** (4.79 g, 20 mmol) in 60 mL of THF at −45 °C. After 1 h of stirring at −40 °C, an oxygen stream was bubbled through the solution for 45 min while the temperature reached −20 °C. The reaction was quenched with water, the product was extracted with $CHCl_3$, and the organic layer was washed with 3% HCl and water until neutral. Triethyl phosphite (3 mL) was added, the organic extracts were dried ($MgSO_4$) and filtered, and the solvent was removed under vacuum. After column chromatography on silica gel (hexane: ethyl acetate = 10:1−10:3.5) and recrystallization from EtOAc/

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hexane, 2.04 g (40%) of pure hydroxy ester were obtained: mp 127−129 °C; $^1$H-NMR $\delta$ 2.21 (3H, s), 2.25 (3H, s), 2.73 (1H, d, $J$ = 6.9 Hz), 2.77 (1H, dd, $J$ = 6.8, 14.7 Hz), 2.89 (1H, dd, $J$ = 4.9, 14.7 Hz), 3.78 (3H, s), 3.81 (3H, s), 4.29 (1H, m, $J$ = 4.9, 6.8, 6.9 Hz), 8.83 (1H, br s) ppm; $^{13}$C-NMR $\delta$ 10.70, 11.59, 29.35, 50.88, 52.36, 71.03, 115.7, 116.9, 127.7, 131.7, 162.2, 175 ppm; IR $\nu$ 3444, 3318, 2952, 1736, 1675, 1509, 1452, 1379, 1274, 1217, 1191, 1088 cm$^{-1}$; MS $m/z$ (rel intens) 255 ($M^{\bullet+}$; 12), 224 (8), 196 (3), 166 (74), 134 (100) amu. Anal. Calcd for $C_{12}H_{17}NO_5$: C, 56.46; H, 6.71; N, 5.49. Found: C, 56.50; H, 6.62; N, 5.42.

**Methyl 3-[2,4-Dimethyl-5-(methoxycarbonyl)-1*H*-pyrrol-3-yl]-2-[(trimethylsilyl)oxy]propionate (3).** To a solution of 2.04 g (8 mmol) of hydroxy ester **2** in 8 mL of dry THF and 48 mL of dry $Et_2O$ was added triethylamine (3.36 mL, 24 mmol) followed by trimethylbromosilane (1.60 mL, 12 mmol). The mixture was stirred for 1 h. The precipitate was filtered and washed with $Et_2O$, and the filtrate was evaporated to dryness. The residue was purified by radial chromatography on silica gel (10−15% acetone in hexane) to afford 2.59 g (99%) of TMS derivative **3**: mp 104−105 °C; $^1$H-NMR $\delta$ −0.05 (9H, s), 2.20 (3H, s), 2.27 (3H, s), 2.72 (1H, *AB*X, $J$ = 8.9, 14.4 Hz), 2.83 (1H, *AB*X, $J$ = 4.4, 14.4 Hz), 3.72 (3H, s), 3.82 (3H, s), 4.14 (1H, AB*X*, $J$ = 4.4, 8.9 Hz), 8.71 (1H, br s) ppm; $^{13}$C-NMR $\delta$ −0.71, 10.63, 11.57, 29.91, 50.81, 51.89, 72.31, 116.6, 116.9, 127.6, 131.6, 162.2, 173.6 ppm; IR $\nu$ 3316, 2951, 1752, 1669, 1508, 1452, 1377, 1273, 1220, 1192, 1132, 1095, 845 cm$^{-1}$. Anal. Calcd for $C_{15}H_{25}NSiO_5$: C, 55.02; H, 7.70; N, 4.28. Found: C, 55.33; H, 7.81; N, 4.27.

**Methyl 3-[(1-(*tert*-Butyloxycarbonyl)-2,4-dimethyl-5-(methoxycarbonyl)-1*H*-pyrrol-3-yl]-2-[(trimethylsilyl)oxy]propionate (4).** To a solution of 2.59 g (7.9 mmol) of pyrrole **3** in 15 mL of $CH_2Cl_2$ and 11 mL (79 mmol) of dry $Et_3N$ was added 97 mg (0.79 mmol) of DMAP followed by 2.07 g (9.5 mmol) di-*tert*-butyl dicarbonate over 10 min. The mixture was stirred for 16 h. The solvents were removed under vacuum, and the residue was purified by radial chromatography on silica gel (7−10% acetone in hexane) to yield 3.17 g (94%) of *N*-(*t*-Boc)-protected pyrrole **4** as a clear oil: $^1$H-NMR $\delta$ −0.04 (9H, s), 1.54 (9H, s), 2.19 (3H, s), 2.27 (3H, s), 2.71 (1H, *AB*X, $J$ = 9.0, 14.4 Hz), 2.82 (1H, *AB*X, $J$ = 4.3, 14.4 Hz), 3.73 (3H, s), 3.81 (3H, s), 4.12 (1H, AB*X*, $J$ = 4.3, 9.0 Hz) ppm; $^{13}$C-NMR $\delta$ −0.72, 10.40, 11.74, 27.47, 29.65, 51.16, 51.94, 72.00, 84.02, 118.1, 120.1, 130.1, 133.8, 149.9, 161.8, 173.3 ppm; IR $\nu$ 2955, 1752, 1713, 1519, 1437, 1373, 1323, 1287, 1252, 1232, 1160, 1105, 844 cm$^{-1}$. Anal. Calcd for $C_{20}H_{33}NSiO_7$: C, 56.18; H, 7.78; N, 3.28. Found: C, 56.35; H, 7.94; N, 3.19.

**Methyl 3-[1-(*tert*-Butyloxycarbonyl)-2,4-dimethyl-5-(methoxycarbonyl)-1*H*-pyrrol-3-yl]-2-hydroxypropionate (5).** To a solution of 3.17 g (7.4 mmol) of pyrrole **4** in 5 mL of dry THF was added 8.9 mL (8.9 mmol) of 1 M tetra-*n*-butylammonium fluoride in THF, and the mixture was stirred for 3 h. It was diluted with 100 mL of $Et_2O$, washed with 50 mL of $H_2O$, dried ($MgSO_4$), and filtered, and the solvent was evaporated under vacuum. The residue was purified by radial chromatography on silica gel (20−25% acetone in hexane) to afford 2.05 g (78%) of α-hydroxy ester **5** as a clear oil: $^1$H-NMR $\delta$ 1.55 (9H, s), 2.17 (3H, s), 2.29 (3H, s), 2.74 (1H, *AB*X, $J$ = 7.3, 14.7 Hz), 2.87 (1H, *AB*X, $J$ = 4.7, 14.7 Hz), 3.79 (3H, s), 3.81 (3H, s), 4.24 (1H, AB*X*, $J$ = 4.7, 7.3 Hz) ppm; $^{13}$C-NMR $\delta$ 10.45, 11.84, 27.54, 29.26, 51.29, 52.54, 70.77, 84.28, 117.2, 120.3, 130.2, 133.9, 149.7, 161.9, 174.6 ppm; IR $\nu$ 3486, 2953, 1746, 1712, 1521, 1440, 1377, 1324, 1289, 1233, 1160, 1101 cm$^{-1}$. Anal. Calcd for $C_{17}H_{25}NO_7$: C, 57.45; H, 7.09; N, 3.94. Found: C, 57.07; H, 7.24; N, 3.80.

**Methyl 3-[2,4-Dimethyl-5-(methoxycarbonyl)-1*H*-pyrrol-3-yl]-2-fluoropropionate (6).** To a cooled to −60 °C solution of 0.75 mL (5.5 mmol) of (diethylamino)sulfur trifluoride in 10 mL of dry $CH_2Cl_2$ was added a solution of 1.78 g (5.0 mmol) of α-hydroxy ester **5** in 8 mL of $CH_2Cl_2$. The temperature was gradually increased over 1 h until it reached ambient. After 30 min more stirring the mixture was diluted with 100 mL of $CH_2Cl_2$, washed with $H_2O$ (3 × 50 mL), dried ($MgSO_4$), and filtered, and the solvent was removed in vacuum. After radial chromatography purification (hexane:acetone = 10:1.5−2), 1.60 g (90%) of clear oil was obtained that contained (by $^1$H- and $^{13}$C-NMR) the desired and rearranged products in a 1.2:1 ratio.

A solution of a 1.60 g mixture of *N*-(*t*-Boc) pyrroles in 3 mL of dry $CH_2Cl_2$ was treated with 2.5 mL of trifluoroacetic acid for 1 h. The mixture was diluted with 100 mL of $CH_2Cl_2$ and washed with saturated aqueous $NaHCO_3$ and $H_2O$ (2 × 100 mL), and after drying and removal of the solvent a quantitative yield of N-deprotected pyrroles (1.2:1 ratio by GC) was obtained. The crude mixture was recrystallized twice from EtOAc/hexane (1:1) to afford 398 mg (31% based on **5** used) of pure isomer **6**: mp 129−130 °C; $^1$H-NMR $\delta$ 2.21 (3H, s), 2.27 (3H, s), 2.94 (1H, m), 3.01 (1H, m), 3.78 (3H, s), 3.82 (3H, s), (4.83, 4.99) (1H, ddd, $^3J$ = 4.5, 7.6 Hz; $^2J_{HF}$ = 48.9 Hz), 8.67 (1H, br s) ppm; $^{13}$C-NMR $\delta$ 10.53, 11.54, (27.41, 27.70) (d, $^2J_{CF}$ = 22.1 Hz), 50.96, 52.35, (87.98, 90.44) (d, $^1J_{CF}$ = 186.3 Hz), (114.8, 114.8) (d, $^3J_{CF}$ = 2.5 Hz), 117.0, 127.6, 131.6, 162.1, (169.8, 170.1) (d, $^2J_{CF}$ = 23.9 Hz) ppm; $^{19}$F-NMR $\delta$ −189.7 (ddd) ppm; IR $\nu$ 3313, 2955, 1743, 1672, 1508, 1453, 1378, 1276, 1212, 1190, 1170, 1098 cm$^{-1}$; MS $m/z$ (rel intens) 257 ($M^{\bullet+}$, 24), 226 (10), 198 (4), 166 (81), 134 (100) amu. $^1$H- and $^{13}$C-NMR spectra of the less crystalline isomer with an α-(fluoromethyl)acetic ester chain (as a mixture with **6**) may be found in the Supporting Information. Anal. Calcd for $C_{12}H_{16}FNO_4$: C, 56.02; H, 6.27; N, 5.44. Found: C, 55.72; H, 6.18; N, 5.34.

**Methyl 2-Fluoro-3-(3-ethyl-2,7,9-trimethyl-1-oxo-1,10-dihydrodipyrrin-8-yl)propanoate (7, Methyl α-Fluoroxanthobilirubinate).** A mixture of 386 mg (1.5 mmol) of monopyrrole **6**, 7.5 mL of EtOH, 300 mg (7.5 mmol) of NaOH, and 3 mL of $H_2O$ was refluxed for 3 h. The solvents were evaporated completely under vacuum. Methanol (10 mL) and 5-(bromomethylene)-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole[26] (324 mg, 1.5 mmol) were added, and the mixture was carefully acidified with concd $HNO_3$. Dry benzene (5 mL) was added, and ∼10 mL were distilled off. The reflux continued for 5 h. The mixture was kept overnight at −20 °C. The precipitate was filtered and purified by radial chromatography (3% $CH_3OH/CH_2Cl_2$) to afford, after recrystallization from $CHCl_3/CH_3OH$, 372 mg (74%) of **7**: mp 210−212 °C; $^1$H-NMR $\delta$ 1.18 (3H, t, $J$ = 7.6 Hz), 1.94 (3H, s), 2.14 (3H, s), 2.41 (3H, s), 2.55 (2H, q, $J$ = 7.6 Hz), (2.97, 3.05) (2 × 1H, 2 × m), 3.80 (3H, s), (4.85, 5.02) (1H, ddd, $^3J$ = 4.6, 7.6 Hz, $^2J_{HF}$ = 49.0 Hz), 6.13 (1H, s), 10.46 (1H, br s), 11.30 (1H, br s) ppm; $^{13}$C-NMR $\delta$ 8.52, 9.68, 11.67, 15.04, 17.94, (27.75, 28.05) (d, $^2J_{CF}$ = 22.2 Hz), 52.36, (88.25, 90.72) (d, $^1J_{CF}$ = 186.3 Hz), 101.0, (113.8, 113.9) (d, $^3J_{CF}$ = 2.2 Hz), 122.6, 122.6, 125.0, 127.4, 132.9, 148.4, (170.0, 170.3) (d, $^2J_{CF}$ = 23.7 Hz), 174.1 ppm; $^{19}$F-NMR $\delta$ −189.3 (ddd) ppm; IR $\nu$ 3339, 3172, 2910, 1753, 1669, 1633, 1468, 1439, 1359, 1271, 1210, 1173, 1085 cm$^{-1}$; UV−vis ($CHCl_3$) $\lambda_{max}$ nm ($\epsilon$) 401 (33 700); ($CH_3OH$) 407 (36 700). Anal. Calcd for $C_{18}H_{23}FN_2O_3$: C, 64.65; H, 6.93; N, 8.38. Found: C, 64.02; H, 6.66; N, 8.12.

**3,17-Diethyl-8,12-bis[2-fluoro-2-(methoxycarbonyl)ethyl]-2,7,13,18-tetramethyl-21*H*,24*H*-biline-1,19-dione (8, α,α′-Difluoromesobiliverdin XIIIα Dimethyl Ester).** A mixture of 334 mg (1 mmol) of dipyrrinone **7**, 615 mg (2.5 mmol) of *p*-chloranil, 220 mL of dry $CH_2Cl_2$, and 11.5 mL of formic acid was heated at reflux for 24 h. The volume was reduced by one half by distillation and reflux continued for an additional 6 h. The mixture was kept overnight at −20 °C, and the separated solid was filtered. The cold filtrate was neutralized with saturated $NaHCO_3$. The organic layer was washed with 5% NaOH (100 mL) and water until neutral (3 × 200 mL), dried ($Na_2SO_4$), and filtered. The solvent was removed under vacuum, and the crude product was purified by radial chromatography (1−1.5% $CH_3OH$ in $CH_2Cl_2$), collecting the nonpolar bright blue band to afford 255 mg (78%) of **8** as mixture of racemic and meso diastereomers: mp 239−243 °C: $^1$H-NMR $\delta$ 1.22 (6H, t, $J$ = 7.7 Hz), 1.82 (6H, s), 2.10 (6H, s), 2.51 (4H, q, $J$ = 7.7 Hz), (3.17, 3.25) (2 × 2H, 2 × m), (3.770, 3.776) (2 × 3H, 2 × s, racemic and meso), (4.98, 5.14) (2 × 1H, ddd, $^3J$ = 4.8, 6.7 Hz, $^2J_{HF}$ = 48.7 Hz), 5.93 (2H, s), 6.64 (1H, s), 8.24 (2H, br s), 9.89 (1H, very br s) ppm; $^{13}$C-NMR $\delta$ 8.28, 9.67, 14.43, 17.80, (27.66, 27.95) (d, $^2J_{CF}$ = 22.2 Hz), 52.59,

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[(87.46, 89.96) (d, $^1J_{CF}$ = 188.8 Hz), (87.55, 90.05 (d, $^1J_{CF}$ = 189.1 Hz) racemic and meso], 96.08, (114.57, 114.6) racemic and meso, 128.5, 129.9, [(131.6, 131.6) (d, $^3J_{CF}$ = 1.3 Hz), 131.73 racemic and meso], 140.15, (141.5, 141.6) racemic and meso, 146.7, 150.0, [(169.4, 169.7) (d, $^2J_{CF}$ = 23.7 Hz), (169.4, 169.7) (d, $^2J_{CF}$ = 23.5 Hz) racemic and meso], 172.7 ppm; $^{19}$F-NMR $\delta$ −188.2 (ddd), −188.7 (ddd) ppm racemic and meso; IR $\nu$ 3310, 2969, 1763, 1699, 1680, 1591, 1455, 1439, 1390, 1271, 1196, 1155, 1098; UV−vis ($CHCl_3$) $\lambda_{max}$ nm ($\epsilon$) 638 (13 300), 364 (46 300); ($CH_3OH$) 640 (13 100), 361 (47 300). Anal. Calcd for $C_{35}H_{40}F_2N_4O_6$: C, 64.60; H, 6.20; N, 8.61. Found: C, 64.41; H, 6.20; N, 8.24.

**8,12-Bis(2-carboxy-2-fluoroethyl)-3,17-diethyl-2,7,13,-18-tetramethyl-10*H*,21*H*,23*H*, 24*H*-biline-1,19-dione (9, α,α′-Difluoromesobilirubin XIIIα).** A mixture of 163 mg (0.25 mmol) of verdin dimethyl ester **8**, 75 mg of ascorbic acid, 75 mL of THF:$CH_3OH$ = 1:1, and 75 mL of 0.2 M aqueous NaOH was stirred at 50 °C for 4 h. After cooling, it was washed with 30 mL of $CHCl_3$, which was discarded. The aqueous layer was cooled with an ice bath and acidified with 10% HCl to pH < 3.5. The blue verdin diacid was extracted with $CHCl_3$ (2 × 100 mL). The solvent from combined extracts was removed under vacuum, and the residual moisture was coevaporated with benzene (2 × 50 mL). The crude solid was used immediately in the next step.

The crude verdin diacid from above was dissolved in 50 mL of dry degassed THF and 20 mL of dry $CH_3OH$. The solution was treated with sodium borohydride (475 mg, 12.5 mmol) added portionwise over 15 min. The reaction was quenched with 100 mL of $H_2O$, cooled to −5 °C, and acidified with 10% HCl until pH < 3.5. The product was extracted with $CHCl_3$, and without washing the solvent was removed under vacuum. The residue was triturated with 2 mL of dry $CH_3OH$ to afford 61 mg (39%) of bright yellow rubin **9**: mp > 320 °C dec; $^{19}$F-NMR $\delta$ −181.8, −184.6, −185.2 ppm; $^1$H- and $^{13}$C-NMR in Table 1; IR (KBr) $\nu$ 3419, 3334, 2966, 2919, 1691, 1658, 1616, 1447, 1353, 1243, 1169, 1076, 975 cm$^{-1}$; UV−vis ($CHCl_3$) $\lambda_{max}$ nm ($\epsilon$) 420 (50 000); ($CH_3OH$) 422 (58 000); FAB-HRMS *m/z* for $C_{33}H_{38}F_2N_4O_6$ calcd 624.2758, found 624.2760.

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**Supporting Information Available:** $^1$H- and $^{13}$C-NMR spectra of compounds **2**−**9** (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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