Altering the Acidity and Solution Properties of Bilirubin. Methoxy and Methylthio Substituents

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Substitution of electron-withdrawing groups at the α-position of an aliphatic carboxylic acid can be expected to increase the acidity of the acid. Methoxy and methylthio groups are especially effective; they increase the acidity of acetic acid by ∼1.1 pK_a units. Bilirubin, the water-insoluble pigment of jaundice, has two propionic acids, and an α-methoxy or α-methylthio substituent in each propionic acid can be expected to lower the pK_a similarly and thus alter its solubility properties. (A previously synthesized analogue, α,α′-difluororubin (**4**), is soluble in water.) Two new analogues of bilirubin, α,α′-dimethoxyrubin (**1**) and α,α′-bis(methylthio)rubin (**2**), have been synthesized, separated into diastereomers, and analyzed. The isomers are shown by NMR to adopt intramolecularly hydrogen-bonded ridge-tile-shaped conformations. Like bilirubin, both **1** and **2** are insoluble in water. Unlike bilirubin, **1** is soluble in dilute aqueous bicarbonate, but **2** is insoluble, which would not be predicted from the expectation that **1** and **2** have the same pK_a. The data hint at a much larger steric size of SCH_3 relative to OCH_3.

Introduction

Bilirubin and biliverdin (Figure 1) are water-insoluble natural pigments produced in adult humans at a rate of ∼300 mg per day by heme catabolism.[1,2] Biliverdin in various forms is widely distributed in nature[3,4] but is not normally detectable in mammals because of its rapid enzymic reduction to bilirubin. Bilirubin has a much narrower distribution, occurring only in mammals. However, it is clinically important for several reasons:[1,2] its accumulation in blood and extravascular tissue is a useful sign of disease, usually liver disease; it can cause irreversible neurologic damage; it is involved in the formation of gallstones; and it may be an important radical-intercepting antioxidant.[5] In addition, bilirubin and its glucuronides have been extensively studied as paradigmatic models for hepatic glucuronidation and for carrier-mediated hepatic uptake and biliary excretion.[6]

Bilirubin is conformationally flexible in solution and preferentially adopts folded conformations shaped like ridge-tiles stabilized by a network of intramolecular hydrogen bonds between the propionic acid carboxyl groups and lactam/pyrrole functions of the neighboring dipyrrinones (Figure 1, inset).[3,7] In contrast, biliverdin and its naturally occurring analogues adopt nonplanar helical conformations resembling a porphyrin when viewed down the helix axis.[3,8] Although bilirubin can form helical conformers similar to those preferred by biliverdin, these are of relatively high energy.[7] The ridge-

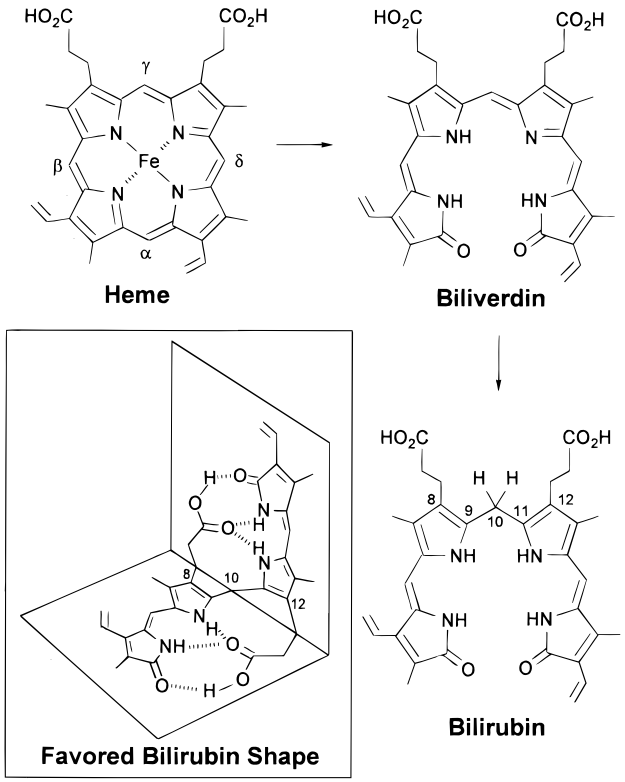

Figure 1. Conversion of heme to bilirubin shown in a prophyrin-like conformation. (Inset) Bilirubin in its energetically most stable, intramolecularly hydrogen-bonded ridge-tile conformation. Only one of two enantiomeric conformers is shown.

(1) (a) McDonagh, A. F. Bile Pigments: Bilatrienes and 5,15-Biladienes. In *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. 6, pp 293−491. (b)Schmid, R.; McDonagh, A. F. Hyperbilirubinemia. In *The Metabolic Basis of Inherited Disease*, 4th ed.; Stanbury, J. B., Wyngaarden, J. B., Fredrickson, D. S., Eds.; McGraw-Hill: New York, 1978; Chapter 51, pp 1221−1257.

(2) Chowdury, J. R.; Wolkoff, A. W.; Chowdury, N. R.; Arias, I. M. Hereditary Jaundice and Disorders of Bilirubin Metabolism. In *The Metabolic and Molecular Bases of Inherited Disease*; Scriver, C. R., Beaudet, A. L., Sly, W. S., Valle, D., Eds.; McGraw-Hill: New York, 1995; Vol. II, Chapter 67, pp 2161−2208.

(3) Falk, H. *The Chemistry of Linear Oligopyrroles and Bile Pigments*; Springer-Verlag: Wien, 1989.

(4) (a) Brittain, T.; Wells, R. M. G. *Comp. Biochem. Physiol.* **1989**, *92B*, 303−306. (b) Fang, L.-S.; Bada, J. L. *Comp. Biochem. Physiol.* **1990**, *97B*, 37−45.

(5) Neuzil, J.; Stocker, R. J. *Biol. Chem.* **1994**, *269*, 16712−16719.

(6) Berk, P. D.; Noyer, C. *Seminars Liver Dis.* **1994**, *14*, 323−394.

(7) Person, R. V.; Peterson, B. R.; Lightner, D. A. *J. Am. Chem. Soc.* **1994**, *116*, 42−59.

(8) Sheldrick, W. S. *Isr. J. Chem.* **1983**, *23*, 155−166.

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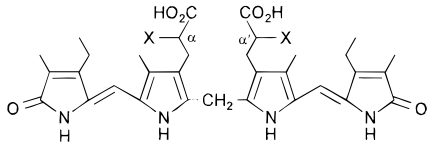
tile conformation is the only one that has been observed in crystals of bilirubin and its carboxylate salts.[9,10,11] Spectroscopic, particularly NMR, studies supported by energy calculations strongly suggest that hydrogen-bonded ridge-tile conformers also prevail in solution, even in the dipolar protophilic solvent dimethyl sulfoxide.[8,12−14] Individual ridge-tile conformers of bilirubin are chiral; both enantiomers occur in solution and interconvert rapidly.[7] Interconversion of the enantiomers occurs via a succession of nonplanar intermediates in which the hydrogen-bonding network is never completely broken.[7,15] The preferred conformation of bilirubin in protein-free aqueous solutions is not known, but calculations and absorption spectra of freshly made dilute solutions suggest that it is similar to that in dimethyl sulfoxide.[15,16]

The acid−base properties of biliverdin and bilirubin are important determinants of the protein and lipid membrane binding behavior of the pigments and of their transport, metabolism, and distribution within organisms. For bilirubin, the acidity (pK_a) of the carboxyl groups is thought to be a key factor in its hepatic transport and neurotoxicity and in the formation of gallstones, which are all poorly understood processes.[17−19] The experimentally determined acidity constants of the propionic carboxyl groups of biliverdin and bilirubin[20−23] are not substantially different from the expected values of pK_a ∼4.5−5.5 shown by other aliphatic carboxylic acids, but carboxylic acid pK_a's in membranes might be expected to be higher.[24,25] Although it is difficult to *increase* the intrinsic pK_a of aliphatic carboxylic acids, it can be *decreased* substantially when electronegative groups are located near the CO_2H group; for example, the pK_a of CH_3CO_2H is 4.76 and that of FCH_2CO_2H is 2.58.[26,27] For bilirubins and their solution properties, such substitution can have very dramatic consequences. Thus, with one fluorine at the α-carbon of each propionic acid, bilirubin analogue **4** is much more polar than its parent, mesobilirubin-XIIIα (**3**), and it is water-soluble.[28] This profound change from the typical hydrophobic bilirubin to one that is hydrophilic is surprising and unexpected. To explore further the relationship between substitution by electronegative groups, carboxylic acid pK_a, and bilirubin solution properties, we synthesized and analyzed two new bilirubin analogues with pK_a values predicted to lie between 2.58 and 4.76: α,α′-dimethoxymesobilirubin-XIIIα (**1**) and α,α′-bis(methylthio)mesobilirubin-XIIIα (**2**). The α-methoxy and α-methylthio substituents are predicted to decrease the propionic acid pK_a less drastically than α-fluoro, to ∼3.7.[26,27]



1: (X=OCH_3) α,α′-Dimethoxymesobilirubin-XIIIα

2: (X=SCH_3) α,α′-Bis(methylthio)mesobilirubin-XIIIα

3: (X=H) Mesobilirubin-XIIIα

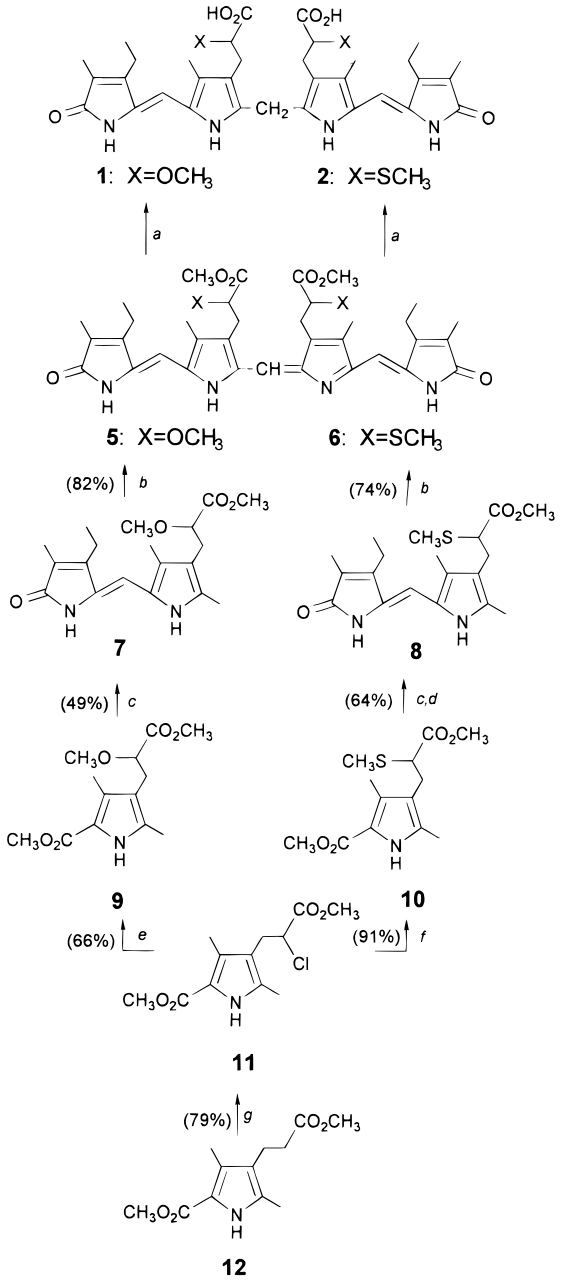
4: (X=F) α,α′-Difluoromesobilirubin-XIIIα

Results and Discussion

Synthesis. Introduction of electron-withdrawing methoxy and methylthio substituents at the propionic acid α-position in mesobilirubin-XIIIα (**3**) was easily achieved at an early stage of the total syntheses. α-Substitution of the propionic ester chain at a later stage, e.g., at the dipyrrole (methyl xanthobilirubinate) or tetrapyrrole (mesobiliverdin-XIIIα dimethyl ester) level, while offering a shorter route, was judged to be intrinsically more difficult synthetically due to the greater sensitivity of these substrates toward the caustic reagents required. We therefore turned to a suitably sturdy known monopyrrole (**12**)[28,29] that was expected to survive functionalization of its propionate chain (Scheme 1). The ester enolate of **12**, prepared by reaction with 2 equiv of LDA, was successfully chlorinated at −78 °C to give **11** using carbon tetrachloride by inverse addition, where the enolate is added to the chlorinating agent.[30] The halogen of **11** was displaced by methoxide or methanethiolate to afford the desired α-methoxy (**9**) or α-methylthio (**10**) pyrrole propionates in 66% and 91% yield, respectively. Diester **9** was saponified, and after acidification, the pyrrole 2-COOH was decarboxylated in situ during condensation (of the resulting 5(*H*)-pyrrole) with 5-bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole[31] in hot methanol to afford bright yellow α-methoxydipyrrinone **7** in 49% yield. (The propionic acid group was simultaneously esterified.) Similarly, treatment of methylthiopyrrole **10** under the same conditions gave mainly the dipyrrinonepropionic acid rather than the ester (**8**). Foretelling interesting differences in properties at the tetrapyrrole stage, the apparently larger steric size of the $-SCH_3$ group seems

(9) Bonnett, R.; Davies, J. E.; Hursthouse, M. B.; Sheldrick, G. M. *Proc. R. Soc. London, Ser. B* **1978**, *202*, 249−268.
(10) LeBas, G.; Allegret, A.; Mauguen, Y.; DeRango, C.; Bailly, M. *Acta Crystallogr., Sect. B* **1980**, *B36*, 3007−3011.
(11) Mugnoli, A.; Manitto, P.; Monti, D. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1983**, *C39*, 1287−1291.
(12) For leading references, see Kaplan, D.; Navon, G. *Isr. J. Chem.* **1983**, *23*, 177−186.
(13) Gawroński, J. K.; Połonski, T.; Lightner, D. A. *Tetrahedron* **1990**, *46*, 8053−8066.
(14) (a) Nogales, D.; Lightner, D. A. *J. Biol. Chem.* **1995**, *270*, 73−77. (b) Dörner, T.; Knipp, B.; Lightner, D. A. *Tetrahedron* **1997**, *53*, 2697−2716.
(15) Shelver, W. L.; Rosenberg, H.; Shelver, W. H. *J. Mol. Struct. (THEOCHEM)* **1994**, *312*, 1−9.
(16) Lee, K.; Garner, L. M. *Pediatr. Res.* **1976**, *10*, 782−788.
(17) Ostrow, J. D.; Mukerjee, P.; Tiribelli, C. *J. Lipid Res.* **1994**, *35*, 1715−1737.
(18) Carey, M. C. *Hepatology* **1984**, *4*, 223S-227S.
(19) Wennberg, R. P. *Pediatr. Res.* **1988**, *23*, 443−447.
(20) Hansen, P. E.; Thiessen, H.; Brodersen, R. *Acta Chem. Scand.* **1979**, *B33*, 281−293.
(21) Lightner, D. A.; Holmes, D. L.; McDonagh, A. F. *Experientia* **1996**, *51*, 639−642.
(22) Holmes, D. L.; McDonagh, A. F.; Lightner, D. A. *J. Biol. Chem.* **1996**, *271*, 2397−2405.
(23) Trull, F. R.; Boiadjiev, S. E.; McDonagh, A. F.; Lightner, D. A. *J. Lipid Res.* **1997**, *38*, 1178−1188.
(24) Hahm, J.-S.; Ostrow, J. D.; Mukerjee, P.; Celic, L. *J. Lipid Res.* **1992**, *33*, 1123−1137.
(25) Hamilton, J. A. ^{13}C NMR Studies of the Interactions of Fatty Acids with Phospholipid Bilayers, Plasma Proteins, and Proteins. In *Carbon-13 NMR Spectroscopy of Biological Systems*; Beckmann, N., Ed.; 1995; Chapter 4, pp 117−157.
(26) Serjeant, E. P.; Dempsey, B. *Ionization Constants of Organic Acids in Aqueous Solution*; Pergamon Press Ltd.: Oxford, 1979, and references therein.
(27) King, J. F. Applications of Dissociation Constants, Optical Activity and Other Physical Measurements (chapter VI In *Elucidation of Structures by Physical and Chemical Methods, Part One*; Bently, K. W., Ed.) In *Techniques of Organic Chemistry*; (Weissberger, A., Ed.; John Wiley & Sons: New York, 1963; Vol. XI, pp 319−412.
(28) Boiadjiev, S. E.; Lightner, D. A. *J. Org. Chem.* **1997**, *62*, 399−404.
(29) Jackson, A. H.; Kenner, G. W.; Sach, G. S. *J. Chem. Soc. (C)* **1967**, 2045−2059.
(30) Boiadjiev, S. E.; Lightner, D. A. *Synlett* **1997**, 1277−1278.
(31) Shrout, D. P.; Lightner, D. A. *Synthesis* **1990**, 1062−1065.

Scheme 1



[a] $NaOH/H_2O$, then HCl, then $NaBH_4/CH_3OH$, then HCl and chromatographic separation: *rac*-**1** (44%), *rac*-**2** (85% of theor.) and *meso*-**2** (34% of theor). [b] *p*-Chloranil, HCOOH. [c] $NaOH/H_2O$, then HNO_3, then react with 5-bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole in refluxing CH_3OH. [d] CH_2N_2. [e] $NaOCH_3$. [f] NaSCH3. [g] LDA, CCl_4.

to inhibit the acid-catalyzed esterification of the propionic acid that attends the condensation reaction. The crude reaction product was treated with diazomethane to afford **8** in 64% yield.

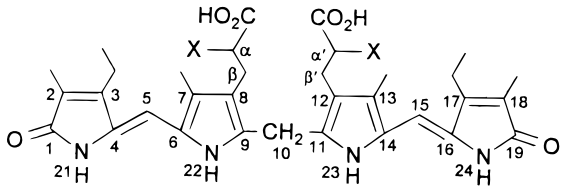
p-Chloranil-promoted oxidative coupling of **7** produced the bright blue α,α′-dimethoxymesobiliverdin-XIIIα dimethyl ester (**5**) in 82% yield. Similar oxidative coupling of **8** gave the deep blue α,α′-dimethylthio analogue (**6**) in 74% yield. NMR analysis showed that both **5** and **6** were unseparated mixtures of the expected α,α′-racemic (*R*,*R* + *S*,*S*) and meso (*R*,*S*) diastereomers in a 1:1 ratio. Mild saponification of these esters gave the corresponding verdin diacids, which without characterization were reduced with sodium borohydride to afford mixtures of the diastereomers of mesobilirubins **1** and **2**. Similar to an earlier observation with α,α′-dimethylmesobilirubin-XIIIα (**13**),[32] the mixture of α,α′-bis(methylthio)mesobilirubins (**2**) could be separated by radial chromatography to afford pure racemic (*rac*-**2**) and meso (*meso*-**2**) diastereomers. In contrast, however, the racemic and meso α,α′-dimethoxymesobilirubin-XIIIα (**1**) exhibited very similar chromatographic mobility, rendering clean chromatographic separation unattainable. After recrystallization of the diastereomeric mixture, *rac*-**1** was obtained (enriched to 85−90% diastereomeric purity).

Spectroscopic Properties. The ^{13}C and ^{1}H NMR spectra of **1** and **2** are compared with the corresponding α,α′-dimethylmesobilirubin-XIIIα (**13**) in Tables 1 and 2. As expected, the ^{13}C NMR data for the racemic diastereomers are very similar, except in the propionic acid chains where the substituents are located. Interestingly, especially strong shieldings of the carboxylic acid carbon of *rac*-**1** and *rac*-**2** are found relative to *rac*-**13**. Although the carbon-13 chemical shifts of *meso*-**13** are very similar to those of *rac*-**13**, those of *meso*-**2** are largely different from those of *rac*-**2**. The signal doubling found in *meso*-**13** is not seen in *meso*-**2**. Such doubling has been attributed to molecular dissymmetry in which one dipyrrinone can engage effectively in intramolecular hydrogen bonding with an opposing propionic acid, but the remaining dipyrrinone cannot, due to a nonbonded steric repulsion between its α-CH_3 and a C(7) or C(13) CH_3.[32] The same sort of nonbonded steric repulsion and molecular dissymmetry might be expected from an α-SCH_3 group, but this does not seem to lead to signal doubling. Yet, *meso*-**2** and *rac*-**2** can be assumed to adopt somewhat different conformations, as significant differences are found in comparing many of the corresponding ^{13}C NMR chemical shifts, especially the lactam carbonyls at C(1) and C(19), the 8^3 and 12^3 CO_2H groups, and the methine carbons at C(5) and C(15), where there is a 3−4 ppm shielding in the *meso* relative to *rac*. Most carbons in *meso*-**2** are more shielded than their counterparts in *rac*-**2**, but it is currently not possible to attribute such variations to specific differences in conformation. Except for the α,α′-CH and α,α′-O(S)CH_3, the carbon chemical shifts of *rac*-**1** and *rac*-**2** are very similar, an indication that they probably adopt the same conformation.

Studies of the ^{1}H NMR spectra of rubins have shown that the pyrrole and lactam NH chemical shifts and the coupling constants within the propionic acid $-CH_2-CH_2-$ or $-CH_2-CHX-$ segment can be particularly useful in expiscating the conformation.[7,12,32,33] Thus in chloroform solvent, the deshielded lactam NH signal near 10.4 ppm in **1** and **2** (Table 2) and relatively more shielded pyrrole NH (near 9 ppm) are characteristic of a dipyrrinone unit of a rubin in a ridge-tile conformation.[7,32−34] In the *meso* diastereomers of **2** and **13**, the signals are doubled, thus indicating a dissymmetry in the ridge-tile. This probably corresponds to one dipyrrinone being tightly hydrogen-bonded intramolecularly to an opposing propionic acid and the second dipyrrinone being engaged in less effective intramolecular hydrogen bonding to the remaining propionic acid (which results in a less deshielded lactam NH

(32) Puzicha, G.; Pu, Y.-M.; Lightner, D. A. *J. Am. Chem. Soc.* **1991**, *113*, 3583−3592.
(33) Boiadjiev, S. E.; Lightner, D. A. *Tetrahedron: Asymmetry* **1997**, *8*, 2115−2129.
(34) Trull, F. R.; Ma, J. S.; Landen, G. L.; Lightner, D. A. *Isr. J. Chem.* **1983**, *23* (2), 211−218.

Table 1. Comparison of ^{13}C NMR Assignments for α,α′-Disubstituted Mesobilirubin-XIIIα Analogues: 1 (X = OCH_3, *rac*), 2 (X = SCH_3, *rac*), 2 (X = SCH_3, *meso*), 13 (X = CH_3, *rac*), and 13 (X = CH_3, *meso*) in $CDCl_3$[a]



position	*rac*-**1** X = OCH_3	*rac*-**2** X = SCH_3	*rac*-**13** X = CH_3	*meso*-**2**[b] X = SCH_3	*meso*-**13** X = CH_3
1,19-CO	174.80	175.02	174.91	171.96	174.89
2,18	124.04	124.04	123.91	123.01	123.92
2,18-CH_3	7.91	7.91	7.95	8.07	7.93
					7.97
3,17	148.61	148.76	148.42	147.24	148.41
3,17-CH_2CH_3	17.83	17.86	17.86	17.16	17.84
3,17-CH_2CH_3	14.88	14.84	14.89	14.84	14.88
4,16	128.48	128.53	128.27	128.01	128.24
					128.84
5,15-CH=	100.92	100.85	100.63	97.63	100.64
6,14	123.34	123.39	123.23	122.03	123.20
7,13	115.96	117.67	117.25	117.21	119.24
7,13-CH_3	10.22	10.32	10.27	9.45	10.26
8,12	124.19	124.43	124.14	123.07	123.94
					124.13
8^1,12^1-CH_2 (β,β′)	26.43	26.40	28.06	25.91	28.04
					29.70
8^2,12^2-CH (α,α′)	79.17	45.11	39.17	47.11	39.14
α,α′-X	58.83	14.72	19.68	13.42	19.18
					19.66
8^3,12^3-COOH	176.64	177.74	182.35	172.87	182.34
9,11	133.58	133.17	133.16	130.95	131.92
					133.15
10-CH_2	22.20	22.12	22.17	23.95	22.69

[a] Values reported for 1×10^{-2} M solutions in ppm downfield from $(CH_3)_4Si$ at 25 °C. [b] In $(CD_3)_2SO$.

(9.6−9.8 ppm) and a less shielded pyrrole NH (9.2−9.4 ppm). The molecular dissymmetry in *meso*-**2** and *meso*-**13** is reflected in signal doubling of most of their proton resonances. The most important distinguishing feature between *rac*-**1**, **2**, or **13** and *meso*-**1**, **2**, or **13** is the appearance of the C(10)−CH_2 signal, which in the C_2-symmetrical racemic diastereomers is a singlet, while in the meso diastereomers it is an AB spin system.

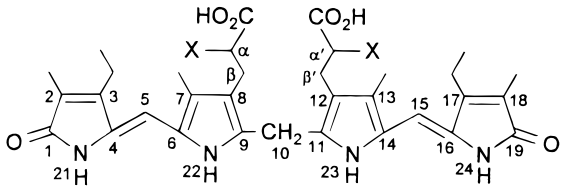
In further support of a well-defined ridge-tile conformation, the vicinal coupling constants in the −CH_2−CHX− segment of the propionic acids follow an ABX pattern characteristic of a fixed staggered chain geometry. Thus, one observes vicinal coupling constants $^3J_{AX}$ = 2−3 and $^3J_{BX}$ = 11−13 Hz in *rac*-**1**, *rac*-**2**, and *rac*-**13** rubins (Table 3). In contrast, in the corresponding monopyrroles **9** and **10**, dipyrrinones **7**, **8**, and **14** (Table 3), or verdins **5** and **6** (all, where intramolecular hydrogen bonding is not possible), one finds only averaged coupling constants ($^3J_{AX}$ = 6−7, $^3J_{BX}$ = 6−9 Hz) characteristic of free rotation in the propionic acid segment. These data strongly suggest a predominantly ridge-tile conformation of these rubins in $CDCl_3$ solvent. In contrast, in $(CD_3)_2$-SO the coupling constants of **1**, **2**, and **13** assume the averaged values typically found in monopyrroles and dipyrrinones in $CDCl_3$: for *rac*-**1** $^3J_{AX}$ = 7.8, $^3J_{BX}$ = 6.1, and $^2J_{AB}$ = 14.3 Hz; for *rac*-**2** $^3J_{AX}$ = 6.6, $^3J_{BX}$ = 9.3, and $^2J_{AB}$ = 14.3 Hz; and for *rac*-**13** $^3J_{AX}$ = 7.7, $^3J_{BX}$ = 7.1−7.3, and $^2J_{AB}$ = 13.8 Hz. These data, however, do not exclude the presence of ridge-tile conformers, and they are consistent with the conclusion of Navon et al.[12] that the propionic acids of bilirubin in $(CD_3)_2SO$ are linked to the opposing dipyrrinones through solvent molecules. Although coupling constants were difficult to determine in the meso diastereomers in $CDCl_3$, the NH chemical shift data support the presence of ridge-tile conformations.

The conclusions reached above are further supported by the $^1H\{^1H\}$ homonuclear Overhauser effects (NOEs)[35] shown in Table 3. Thus, NOEs observed between the lactam and pyrrole NH's and between the C(5)/C(15) hydrogens and the C(7)/C(13) methyls and the C(3^1)/C(17^1) methylenes confirm a *syn*-periplanar geometry about the exocyclic 4*Z*,15*Z* double bonds of *rac*-**1** and *rac*-**2**. Most importantly, the propionic acid α,α′-methine hydrogens showed a very strong NOE on the C(7)/C(13) methyls. This implies a close proximity between the α,α′-CH's and the C(7)/C(13) CH_3's, as is characteristically found only in the ridge-tile conformation. Consistent with this picture, irradiation of the α,α′-methine hydrogens produced a strong NOE on the β,β′-H at 2.80 (2.78) ppm (H_A) and a much weaker NOE on the remaining β,β′-H at 3.16 (3.12) ppm (H_B) (Figure 2). Taken collectively, the NOE data together with propionic acid chain coupling constants confirm the fixed staggered geometry represented in Table 3, which follows from the ridge-tile structures of *rac*-**1**, *rac*-**2**, and *rac*-**13** that are very similar to that of bilirubin (shown in Figure 1, inset).

Interestingly, although the carboxylic acid protons of **2** and **13** could be detected easily in $CDCl_3$ (and $(CD_3)_2$-SO) solvent at room temperature (Figure 3), they could

(35) (a) Stonehouse, J.; Adell, P.; Keeler, J.; Shaka, A. J. *J. Am. Chem. Soc.* **1994**, *116*, 6037−6038. (b) Stott, K.; Keeler, J.; Van, Q. N.; Shaka, A. J. *J. Magn. Reson.* **1997**, *125*, 302−324.

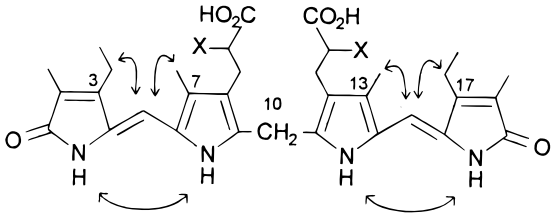
Table 2. ^{1}H NMR Chemical Shift Assignments for α,α′-Disubstituted Mesobilirubin-XIIIα analogues: 1 (X = OCH_3, *rac*), 2 (X = SCH_3, *rac*), 2 (X = SCH_3, *meso*), 13 (X = CH_3, *rac*), and 13 (X = CH_3, *meso*) in $CDCl_3$ Solutions[a]

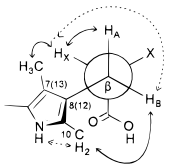


position	*rac*-**1** X = OCH_3	*rac*-**2** X = SCH_3	*meso*-**2** X = SCH_3	*rac*-**13** X = CH_3	*meso*-**13** X = CH_3
α,α′-COOH	14.8[b]	14.3	14.5	13.7	13.7
21,24-NHCO	10.38	10.41	9.61	10.55	9.84
			10.52		10.54
22,23-NH	9.01	8.96	8.88	9.09	9.09
			9.20		9.37
2,18-CH_3	1.86	1.86	1.86	1.86	1.86
3,17-CH_2CH$_3$	2.48[c]	2.49[c]	2.49[c]	2.48[c]	2.48[c]
3,17-CH$_2$CH_3	1.12[d]	1.13[d]	1.13[d]	1.12[d]	1.12[d]
5,15-CH=	6.06	6.06	6.06	6.05	6.05
			6.10		6.07
7,13-CH_3	2.18	2.19	2.18	2.15	2.14
			2.22		2.15
8^2,12^2-CH (α,α′)	4.16[e]	3.71[h]	3.72[k]	3.05[m]	3.03[k]
					3.05[k]
α,α′-X	3.66	2.36	2.32	1.45[n]	1.45[n]
			2.33		1.46[n]
8^1,12^1-CH_2 (β,β′)	2.80[f]	2.78[i]	2.73[k]	2.42[o]	2.42[q]
	3.16[g]	3.12[j]	3.21[k]	2.90[p]	2.90[r]
10-CH_2	4.02	4.00	4.00[l]	4.04	4.04[l]
			4.08[l]		4.08[l]

[a] Values reported in ppm downfield from $(CH_3)_4Si$ at 25 °C for 1×10^{-3} M solutions. [b] At −50 °C. [c] q, $J = 7.6$ Hz. [d] t, $J = 7.6$ Hz. [e] AB*X*, $^3J = 3.4$, 11.4 Hz. [f] ABX, $^3J = 3.4$ Hz, $^2J = 14.2$ Hz. [g] ABX, $^3J = 11.4$ Hz, $^2J = 14.2$ Hz. [h] AB*X*, $^3J = 3.6$, 13.0 Hz. [i] ABX, $^3J = 3.6$ Hz, $^2J = 14.6$ Hz. [j] AB*X*, $^3J = 13.0$ Hz, $^2J = 14.6$ Hz. [k] br m. [l] AB, $^2J = 15.6$ Hz. [m] AB*X*, $^3J = 2.2$, 12.2 Hz. [n] d, $J = 7.1$ Hz. [o] ABX, $^3J = 2.2$ Hz, $^2J = 14.4$ Hz. [p] AB*X*, $^3J = 12.2$ Hz, $^2J = 14.4$ Hz. [q] ABX, $^3J = 2.9$ Hz, $^2J = 14.5$ Hz. [r] AB*X*, $^3J = 12.1$ Hz, $^2J = 14.5$ Hz.

Table 3. Coupling Constants[a] for Hydrogens in the Propionic Acids at C(8) or C(12) and Relevant ^{1}H{^{1}H} Homonuclear Overhauser Effects (Indicated by Curved Arrows) of Bilirubin Analogs 1, 2, and 13; Dipyrrinones 7 and 8, and Their α-Methyl Analogue 14 in $CDCl_3$[b]



Fixed Staggered Propionic Acid Geometry	Rubin (X)	Chemical Shift[a] δ_A	δ_B	δ_X	Coupling Constant[a] $^3J_{AX}$	$^3J_{BX}$	$^2J_{AB}$
	*rac*-**1** (OCH_3)	2.80	3.16	4.16	3.4	11.4	14.2
	rac-**2** (SCH_3)	2.78	3.12	3.71	3.6	13.0	14.6
	rac-**13** (CH_3)	2.42	2.90	3.05	2.2	12.2	14.4
	7, (OCH_3)	2.82	2.86	3.82	7.4	5.9	14.6
	8, (SCH_3)	2.77	3.05	3.38	6.6	8.9	14.6
	14, (CH_3)[c]	2.46	2.81	2.59	8.7	6.3	14.2

[a] *J* values in Hz; δ in ppm downfield from $(CH_3)_4Si$ at 25 °C. [b] NOEs indicated on the partial structures: solid curved arrows indicate strong NOEs; dotted curved arrows indicate weaker NOEs. [c] Methyl α-methylxanthobilirubinate (**14**), the α-methyl analogue of **7** and **8**.

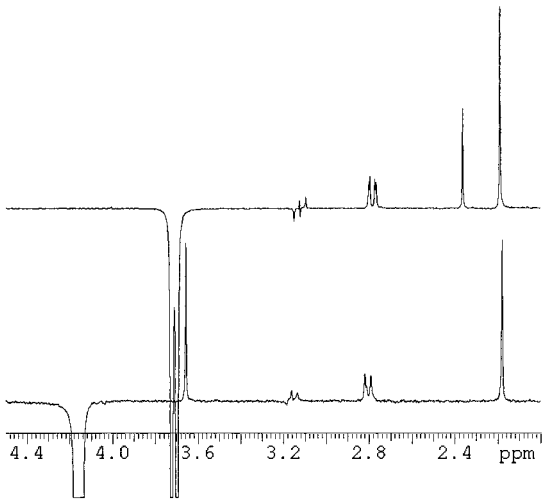


Figure 2. ^{1}H{^{1}H} NOEs in *rac*-**1** (lower) and *rac*-**2** (upper) in $CDCl_3$ found when irradiating the propionic acid α,α′-methine hydrogens. A strong NOE is seen for the C(7)/C(13) methyl groups at 2.18 ppm in *rac*-**1** and 2.19 ppm in *rac*-**2** as well as for the *syn* β,β′-H's at 2.80 ppm in *rac*-**1** and 2.78 ppm in *rac*-**2**, while only very weak NOEs are seen for the *anti* β,β′-H's at 3.16 and 3.12 ppm.

not be detected in **1** in $CDCl_3$ until the temperature was lowered to −50 °C (Figure 4). There were no significant changes in the remainder of the ^{1}H NMR spectra of *rac*-**1** or *rac*-**2** down to −50 °C, or even at +50 °C, where the propionic acid coupling constants (Table 3) also remained unchanged. The last is particularly interesting, as it suggests a higher activation barrier for conformational inversion than that of bilirubin;[7,12] that is, the ridge-tile conformations of *rac*-**1**, *rac*-**2**, and *rac*-**13** are more stable than those of bilirubin or the parent **3**.

The UV−vis spectral data (Table 4) for **1** and **2** differ only slightly from those of **13** or from those of their α,α′-difluoro analogue (**4**) and the parent, mesobilirubin-XIIIα

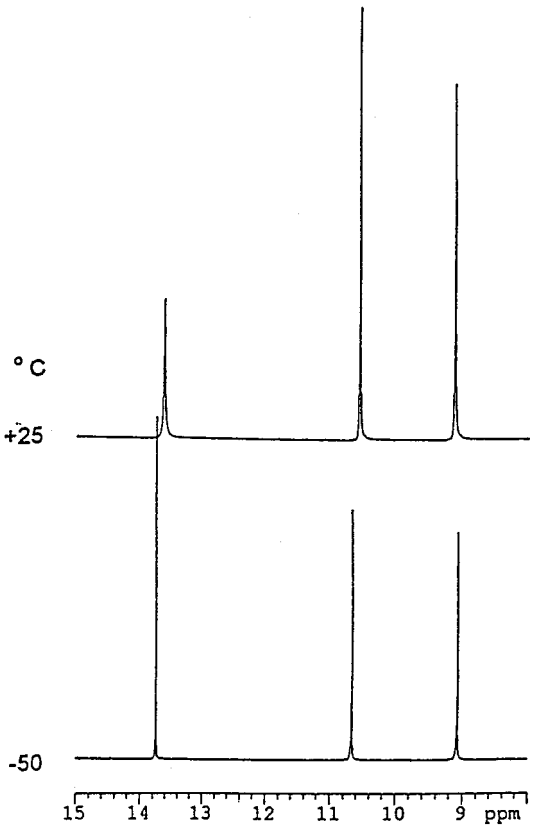


Figure 3. CO_2H and NH resonances of 10^{-3} M *rac*-**2** at +25 and −50 °C in $CDCl_3$.

(**3**).[28] Since the UV−vis spectra originate from exciton coupling between the two dipyrrinone chromophores of the rubin, they can be expected to be sensitive to the relative orientation of the chromophores,[7] hence the conformation of the pigment. The considerable similarity in λ_{max} of the various pigments in Table 4 suggests that this conformation is rather insensitive to solvent. As with the NMR data, these spectral data too are consistent with a ridge-tile-shaped conformer.

When bound to human serum albumin, bilirubin is known to exhibit a strong induced circular dichroism (CD), with exciton coupling exhibited by the bisignate Cotton effects near 400 nm.[36,37] The phenomenon is general with bilirubin analogues that preferentially adopt helical conformations[38] such as in Figure 1 (inset) and has been observed with other proteins[36] and with other chiral complexation agents.[39] The chiral complexation agent, in the current study human serum albumin (HSA), is thought to unbalance the conformational enantiomer equilibrium shown in Figure 5 (inset) and lead to CD observed for the ~400 nm UV−vis transitions (Table 4). The pH 7.4 buffered HSA solutions of *rac*-**1** give especially strong CD spectra, stronger than the parent rubin (**3**) or α,α′-difluororubin **4**. Thus, the α,α′-methoxy groups appear to be especially potent in facilitating displacement of the M ⇌ P conformational equilibrium when the rubin **1** forms an association complex with HSA. According to exciton chirality theory,[7,40] the observed exciton couplet with long-wavelength positive, short-wavelength negative Cotton effect indicates a predominance of the P-chirality. Surprisingly, the CD Cotton effects of *rac*-**2** are much weaker than those of *rac*-**1**, or even the parent (**3**), while those of *rac*-**13** are weak and oppositely signed from all of the others. It is not clear why the presence of α,α′-methylthio and α,α′-methyl groups cause such weak CD spectra while α,α′-methoxy groups considerably enhance the Cotton effects observed with the parent (**3**).

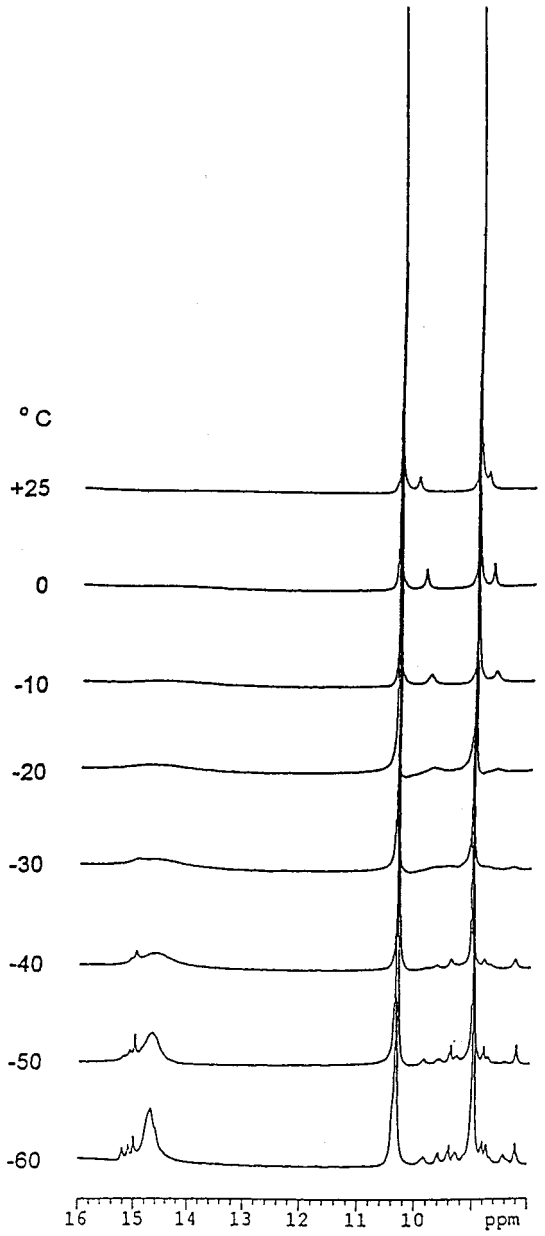
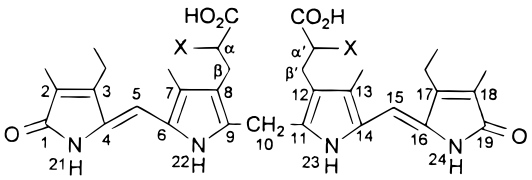


Figure 4. CO_2H and NH resonances of 10^{-3} M *rac*-**1** in ^1H NMR spectra recorded between +25 and −60 °C in $CDCl_3$.

(36) Blauer, G. *Isr. J. Chem.* **1983**, *23*, 201−209.
(37) (a) Lightner, D. A.; Wijekoon, W. M. D.; Zhang, M. H. *J. Biol. Chem.* **1988**, *263*, 16669−16676. (b) Pu, Y.-M.; McDonagh, A. F.; Lightner, D. A. *J. Am. Chem. Soc.* **1993**, *115*, 377−380.
(38) (a) Krois, D.; Lehner, H. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1837−1840. (b) Krois, D.; Lehner, H. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1351−1360.
(39) (a) Trull, F. R.; Shrout, D. P.; Lightner, D. A. *Tetrahedron* **1992**, *48*, 8189−8198. (b) Lightner, D. A.; An, J. Y.; Pu, Y. M. *Arch. Biochem. Biophys.* **1988**, *262*, 543−559. (c) Lightner, D. A.; Gawroński, J. K.; Wijekoon, W. M. D. *J. Am. Chem. Soc.* **1987**, *109*, 6354−6362. (d) Reisinger, M.; Lightner, D. A. *J. Inclusion Phenomena* **1985**, *3*, 479−486. (e) Lightner, D. A.; Gawroński, J. K.; Gawrońska, K. *J. Am. Chem. Soc.* **1985**, *107*, 2456−2461.
(40) Harada, N.; Nakanishi, K. *Circular Dichroism Spectroscopy-Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.

Table 4. Comparison of UV−Vis Spectral Data of α,α′-Dimethoxy (1), Bis(methylthio) (2), and Dimethyl (13) mesobilirubin-XIIIα Analogues and the Parent (3) at 22 °C[a]



pigment, X =	solvent	ϵ^{max} [b]	(λ, nm)	ϵ_{sh} [b,c]	(λ, nm)
rac-**1**, OCH_3	benzene	55 800	(433)	53 200	(418)
rac-**2**, SCH_3		54 200	(431)	51 700	(416)
meso-**2**, SCH_3		56 400	(429)	54 000	(411)
rac-**13**, CH_3		56 300	(431)	50 900	(417)
meso-**13**, CH_3		57 200	(431)	54 600	(412)
3, H		58 800	(435)	54 700	(417)
rac-**1**, OCH_3	$CHCl_3$	54 100	(425)		
rac-**2**, SCH_3		54 000	(431)		
meso-**2**, SCH_3		53 800	(426)	52 500	(410)
rac-**13**, CH_3		56 100	(433)		
meso-**13**, CH_3		55 900	(425)		
3, H		57 800	(431)		
rac-**1**, OCH_3	CH_3OH	58 800	(424)		
rac-**2**, SCH_3		55 800	(422)		
meso-**2**, SCH_3		62 300	(427)		
rac-**13**, CH_3		55 700	(426)		
meso-**13**, CH_3		57 400	(426)		
3, H		50 700	(426)	43 100	(401)
rac-**1**, OCH_3	CH_3CN	54 100	(417)		
rac-**2**, SCH_3		52 700	(417)		
meso-**2**, SCH_3		59 700	(420)	57 600	(402)
rac-**13**, CH_3		55 200	(425)		
meso-**13**, CH_3		58 300	(420)		
3, H		56 700	(422)		
rac-**1**, OCH_3	$(CH_3)_2SO$	58 700	(425)	53 300	(398)
rac-**2**, SCH_3		58 500	(421)	50 600	(396)
meso-**2**, SCH_3		66 800	(430)	60 500	(410)
rac-**13**, CH_3		52 800	(426)	47 300	(407)
meso-**13**, CH_3		61 300	(428)	50 000	(398)
3, H		57 000	(426)	49 100	(397)

[a] Conc ~1.5×10^{-5} M. [b] ϵ in L mol^{-1} cm^{-1}. [c] sh = shoulder.

Solution Properties. Like bilirubin and mesobilirubin-XIIIα (**3**), **1** and **2** are insoluble in water, whereas **4** is water-soluble. Apparently, solubility in neutral pH water can be achieved when the substituent causes the propionic acid pK_a to drop to ~2.6 but not to ~3.7. Whether the methyl groups in the OCH_3 and SCH_3 substituents play a role in determining water insolubility is unclear but might be resolved from an examination of a rubin with α-OH substituents. Like the parent, *rac*-**2** is insoluble in dilute aqueous sodium bicarbonate, but *rac*-**1** is soluble. This is odd, considering that the acidities of α-methoxy and α-(methylthio)acetic acid are nearly the same[26,27] (pK_a ~3.7). Also like bilirubin, the racemic diastereomers *rac*-**1** and *rac*-**2** are soluble in chloroform but insoluble in methanol. This again contrasts with the behavior of either diastereomer of the α,α′-difluororubin (**4**), which are insoluble in chloroform and soluble in methanol. Qualitatively, *rac*-**2** is more soluble in chloroform and less soluble in methanol than *rac*-**1**. Interestingly, the polarity difference between *rac*-**2** and *meso*-**2** is much greater than between *rac*-**1** and *meso*-**1**, so much so that while the diastereomers of **2** could be separated by adsorption chromatography, those of **1** could not. The R_f values for the rubins on silica gel TLC using 1% methanol in dichloromethane are *rac*-**1** (0.07), *rac*-**2** (0.87), *meso*-**2** (0.36), **3** (0.76), **4** (0.00), *rac*-**13** (0.84), and *meso*-**13** (0.46). Significantly, the racemic and meso diastereomers of **2** exhibit very different solubility properties: like bilirubin, *rac*-**2** is insoluble in methanol but freely soluble in chloroform, but *meso*-**2** is more soluble in methanol and much less soluble in chloroform. The solubility properties of **1** and **2** are consistent with intramolecularly hydrogen-bonded conformations (Figure 1 (inset)).

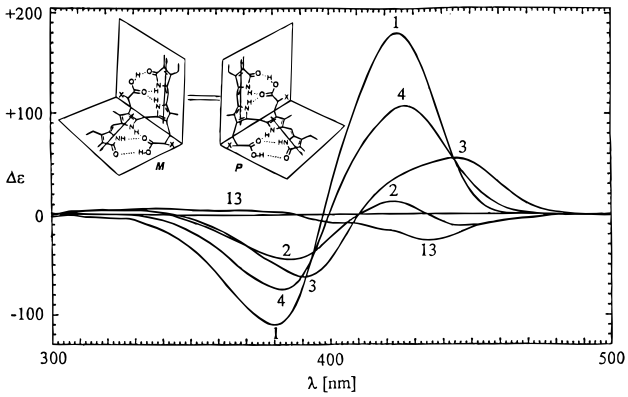


Figure 5. Circular dichroism of *rac*-**1** (X = OCH_3, curve 1), *rac*-**2** (X = SCH_3, curve 2), *rac*-**13** (X = CH_3, curve 13), **3** (X = H, curve 3), and **4** (X = F, curve 4) on human serum albumin (HSA) in pH 7.4 phosphate buffer at 22 °C. The concentration of pigment is ~2×10^{-5} M and that of HSA is 4×10^{-5} M. (Inset) Interconverting intramolecularly hydrogen-bonded enantiomeric conformations of mesobilirubins (**1**−**4**). The double-headed arrows represent the dipyrrinone long-wavelength electric dipole transition moments (vectors), and the relative helicities of the vectors are given as M, minus, or P, plus.

Concluding Comments

Substitution of methoxy and methylthio groups at the α-carbon of the propionic acid side chains of bilirubin is expected to lead to the same reduced pK_a and similar solution properties. Synthetic analogues **1** (α,α′-dimethoxymesobilirubin-XIIIα) and **2** (α,α′-bis(methylthio)mesobilirubin-XIIIα) behave differently, however. Although neither are soluble in water, like α,α′-difluoromesobilirubin-XIIIα (**4**), *rac*-**1** is soluble in dilute bicarbonate, while *rac*-**2** is insoluble. Like bilirubin, both **1** and **2** are soluble in chloroform and insoluble in methanol, and NMR spectroscopic analysis confirms that the pigments adopt intramolecularly hydrogen-bonded conformations shaped like ridge-tiles. Very large induced Cotton effects are found in the circular dichroism spectra of **1** in pH 7.4 phosphate buffered aqueous human serum albumin (HSA). Much weaker Cotton effects are found for **2**. Thus, although the same pK_a values are predicted for **1** and **2** and they adopt similar conformations, their solubility in dilute base and their complexation with HSA differ. The data, taken collectively, suggest a larger steric size of SCH_3 over OCH_3.

Experimental Section

NMR spectra were obtained at 300 and 500 MHz in $CDCl_3$ solvent (unless otherwise noted), and chemical shifts were reported in δ ppm. Analytical thin-layer chromatography (TLC), radial chromatography, and HPLC analyses were carried out as reported previously.[28] Melting points are uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ. Dimethyl malonate, pentane-2,4-dione, methyl methacrylate, diisopropylamine, *n*-butyllithium in hexane, *p*-chloranil, and sodium borohydride were from Ald-

rich. Methanethiol was from Pfaltz and Bauer. 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 $Mg(OCH_3)_2$; and 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)propanoate (**12**) was prepared as reported previously.[28,29]

Methyl 2-Chloro-3-(2,4-dimethyl-5-methoxycarbonyl-1*H*-pyrrol-3-yl)propanoate (**11**). A solution of 10.80 g (45 mmol) pyrrole **12** in 150 mL of dry THF was added via syringe to a N_2-protected solution of LDA (freshly prepared from dry diisopropylamine (14 mL, 100 mmol) in THF (150 mL) and 2.5 M *n*-butyllithium in hexane (39.5 mL, 99 mmol)) at −60 to −50 °C. After stirring for 1 h at −60 °C the solution was cooled to −78 °C and transferred using a double-tipped needle under N_2 pressure to a solution of CCl_4 (8.7 mL, 90 mmol) in THF (80 mL) kept at −78 °C. After stirring for 1 h at −78 °C the reaction was quenched with saturated aqueous NH_4Cl, diluted with 500 mL of CH_2Cl_2, and washed with 2% HCl (100 mL) and water (3 × 200 mL). The organic phase was dried (anhyd $MgSO_4$), filtered, and evaporated under vacuum. Recrystallization from CH_3OH/H_2O (8:2) afforded 9.74 g (79%) of α-chloroester **11**: mp 123−124 °C; ^{1}H NMR ($CDCl_3$) δ 2.23 (3H, s), 2.27 (3H, s), 2.97 (1H, dd, ^3J = 7.6 Hz, ^2J = 14.7 Hz), 3.15 (1H, dd, ^3J = 7.6 Hz, ^2J = 14.7 Hz), 3.74 (3H, s), 3.82 (3H, s), 4.29 (1H, dd, ^3J = 7.6, 7.6 Hz), 8.81 (1H, br s) ppm; ^{13}C NMR ($CDCl_3$) δ 10.63, 11.56, 30.22, 50.98, 52.86, 56.49, 115.72, 117.11, 127.26, 131.62, 162.15, 169.95 ppm; IR ν 3303, 2955, 1743, 1672, 1510, 1452, 1380, 1277, 1212, 1194, 1170, 1096 cm^{-1}; MS *m*/*z* (rel int) 275, 273 ($M^{\bullet+}$, 14), 244, 242 (6), 206 (4), 166 (96), 134 (100) amu. Anal. Calcd for $C_{12}H_{16}ClNO_4$ (273.7): C, 52.65; H, 5.89; N, 5.12. Found: C, 52.53; H, 6.02; N, 5.03.

Methyl 3-(2,4-Dimethyl-5-methoxycarbonyl-1*H*-pyrrol-3-yl)-2-methoxypropanoate (9). To 140 mL of dry methanol was slowly added 2.30 g (100 mg atoms) of sodium under N_2 with occasional cooling using an ice bath. After all the sodium had reacted, a solution of 10.94 g (40 mmol) of α-chloroester **11** in 200 mL of dry methanol was added, and the mixture was heated at reflux for 40 min. It was cooled to 0 °C; then 300 mL of cold CH_2Cl_2 was added, and the mixture was washed with ice cold water (5 × 200 mL) until neutral. The organic layer was dried (anhyd Na_2SO_4) and filtered, and the solvent was evaporated under vacuum. The residue was recrystallized from 40 mL of methanol to afford 7.10 g (66%) of α-methoxy ester **9**: mp 136−137 °C; ^{1}H NMR ($CDCl_3$) δ 2.21 (3H, s), 2.27 (3H, s), 2.78 (1H, dd, ^3J = 7.4 Hz, ^2J = 14.6 Hz), 2.81 (1H, dd, ^3J = 5.9 Hz, ^2J = 14.6 Hz), 3.32 (3H, s), 3.73 (3H, s), 3.78 (1H, dd, ^3J = 5.9, 7.4 Hz), 3.82 (3H, s), 8.60 (1H, br s) ppm; ^{13}C NMR ($CDCl_3$) δ 10.57, 11.55, 28.07, 50.84, 51.86, 58.29, 81.44, 116.50, 116.70, 127.61, 131.45, 162.10, 172.81 ppm; IR ν 3290, 2953, 2829, 1747, 1686, 1501, 1460, 1372, 1277, 1225, 1187, 1122, 1025 cm^{-1}; MS *m*/*z* (rel int) 269 ($M^{\bullet+}$; 17), 238 (7), 210 (8), 166 (79), 134 (100) amu. Anal. Calcd for $C_{13}H_{19}NO_5$ (269.3): C, 57.98; H, 7.11; N, 5.20. Found: C, 57.86; H, 7.13; N, 5.18.

Methyl 3-(2,4-Dimethyl-5-methoxycarbonyl-1*H*-pyrrol-3-yl)-2-(methylthio)propanoate (10). Sodium (1.73 g, 75 mg atoms) was added slowly under nitrogen to 80 mL of dry methanol cooled with an ice bath. After all the sodium had reacted, methanethiol (**Caution!** highly toxic, stench) was bubbled through the mixture at 0 °C for 15 min (until a yellow precipitate appeared in the 15 cm long trap filled with saturated aqueous $Pb(OAc)_2$ and attached to the condenser effluent end). A solution of 8.21 g (30 mmol) of α-chloroester **11** in 90 mL of dry methanol was added, and the mixture was heated at reflux for 35 min. It was cooled to 0 °C; then 300 mL of cold CH_2Cl_2 was added, and the mixture was washed with cold water (4 × 200 mL). The organic layer was dried (anhyd Na_2SO_4) and filtered, and the solvent was evaporated under vacuum. The residue was recrystallized from 50 mL of methanol to afford 7.80 g (91%) of α-methylthioester **10**: mp 130−131 °C; ^{1}H NMR ($CDCl_3$) δ 2.15 (3H, s), 2.22 (3H, s), 2.28 (3H, s), 2.73 (1H, dd, ^3J = 6.6 Hz, ^2J = 14.6 Hz), 3.00 (1H, dd, ^3J = 8.9 Hz, ^2J = 14.6 Hz), 3.34 (1H, dd, ^3J = 6.6, 8.9 Hz), 3.68 (3H, s), 3.82 (3H, s), 8.58 (1H, br s) ppm; ^{13}C NMR ($CDCl_3$) δ 10.63, 11.45, 14.18, 26.18, 48.01, 50.81, 51.99, 116.83, 117.55, 127.17, 131.16, 162.19, 172.33 ppm; IR ν 3307, 2951, 2918, 2856, 1731, 1670, 1507, 1450, 1378, 1338, 1271, 1220, 1189, 1156, 1091 cm^{-1}; MS *m*/*z* (rel int) 285 ($M^{\bullet+}$; 20), 254 (6), 226 (5), 166 (100), 134 (91) amu. Anal. Calcd for $C_{13}H_{19}NO_4S$ (285.4): C, 54.71; H, 6.71; N, 4.91. Found: C, 55.01; H, 6.82; N, 4.90.

Methyl 3-(3-Ethyl-2,7,9-trimethyl-1-oxo-1,10-dihydrodipyrrin-8-yl)-2-methoxypropanoate (7, Methyl α-Methoxyxanthobilirubinate). A mixture of 1.35 g (5 mmol) of monopyrrole **9**, 1.00 g (25 mmol) of NaOH, 25 mL of EtOH, and 10 mL of H_2O was heated at reflux for 3 h. The solvents were then evaporated completely under vacuum. To the residue was added dry methanol (50 mL), and the solution was carefully acidified with concentrated HNO_3. 5-Bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole[31] was added, and the mixture was brought to reflux. To remove water, two successive 30 mL portions of 1:1 (by vol) dry benzene and methanol were added, and after each addition, 30 mL was removed by distillation. Reflux was continued for 5 h; then the reaction was cooled, and the mixture was kept overnight at −20 °C. The resulting precipitate was collected by filtration and purified by radial chromatography (2.5−4% CH_3OH in CH_2Cl_2) to afford (after recrystallization from $CHCl_3$−CH_3OH) 855 mg (49%) of dipyrrinone **7**: mp 207−208 °C; ^{1}H NMR ($CDCl_3$) δ 1.18 (3H, t, ^3J = 7.6 Hz), 1.95 (3H, s), 2.15 (3H, s), 2.41 (3H, s), 2.55 (2H, q, ^3J = 7.6 Hz), 2.82 (1H, dd, ^3J = 7.4 Hz, ^2J = 14.6 Hz), 2.86 (1H, dd, ^3J = 5.9 Hz, ^2J = 14.6 Hz), 3.34 (3H, s), 3.74 (3H, s), 3.82 (1H, dd, ^3J = 5.9, 7.4 Hz), 6.14 (1H, s), 10.38 (1H, br s), 11.26 (1H, br s) ppm; ^{13}C NMR ($CDCl_3$) δ 8.52, 9.72, 11.70, 15.04, 17.94, 28.41, 51.91, 58.37, 81.73, 101.23, 115.59, 122.36, 122.36, 125.33, 127.02, 132.92, 148.33, 173.03, 174.08 ppm; IR ν 3348, 3191, 2920, 2826, 1750, 1666, 1635, 1465, 1435, 1371, 1271, 1198, 1174, 1118, 1027 cm^{-1}; UV−vis λ_{max} nm (ϵ) ($CHCl_3$) 405 (34 700), (CH_3OH) 409 (38 500), ($(CH_3)_2SO$) 410 (37 100). Anal. Calcd for $C_{19}H_{26}N_2O_4$ (346.4): C, 65.87; H, 7.57; N, 8.09. Found: C, 65.62; H, 7.29; N, 7.99.

Methyl 3-(3-Ethyl-2,7,9-trimethyl-1-oxo-1,10-dihydrodipyrrin-8-yl)-2-(methylthio)propanoate (8, Methyl α-(Methylthio)xanthobilirubinate). Preparation of **8** from 5 mmol of **10** followed as above for **7,** except after 5.5 h reflux the crude product was extracted into $CHCl_3$, washed with H_2O until neutral, and treated in THF−CH_3OH with excess diazomethane. Thus, 5 mmols of monopyrrole **10** afforded 1.154 g (64%) of bright yellow dipyrrinone **8**: mp 221−222 °C; ^{1}H NMR ($CDCl_3$) δ 1.18 (3H, t, ^3J = 7.6 Hz), 1.94 (3H, s), 2.15 (3H, s), 2.17 (3H, s), 2.42 (3H, s), 2.55 (2H, q, ^3J = 7.6 Hz), 2.77 (1H, dd, ^3J = 6.6 Hz, ^2J = 14.6 Hz), 3.05 (1H, dd, ^3J = 8.9 Hz, ^2J = 14.6 Hz), 3.38 (1H, dd, ^3J = 6.6, 8.9 Hz), 3.70 (3H, s), 6.12 (1H, s), 10.39 (1H, br s), 11.30 (1H, br s) ppm; ^{13}C NMR ($CDCl_3$) δ 8.50, 9.75, 11.77, 14.29, 15.01, 17.91, 26.52, 48.20, 52.10, 101.06, 116.72, 122.44, 122.51, 124.84, 127.14, 132.48, 148.33, 172.53, 174.07 ppm; IR ν 3338, 3139, 2959, 2873, 1736, 1666, 1635, 1463, 1435, 1378, 1346, 1313, 1274, 1230, 1172, 1157 cm^{-1}; UV−vis λ_{max} nm (ϵ) ($CHCl_3$) 405 (35 400), (CH_3OH) 409 (39 100), ($(CH_3)_2SO$) 409 (36 400). Anal. Calcd for $C_{19}H_{26}N_2O_3S$ (362.5): C, 62.95; H, 7.23; N, 7.73. Found: C, 62.92; H, 7.30; N, 7.68.

3,17-Diethyl-8,12-bis[2-methoxy-2-(methoxycarbonyl)ethyl]-2,7,13,18-tetramethyl-21*H*,24*H*-bilin-1,19-dione (5, α,α′-Dimethoxymesobiliverdin-XIIIα Dimethyl Ester). A mixture of 346 mg (1 mmol) of dipyrrinone **7**, 615 mg (2.5 mmol) of *p*-chloranil, 220 mL of dry CH_2Cl_2, and 11 mL of formic acid was heated at reflux for 24 h. The volume was reduced by one-half by distillation, and reflux was continued for an additional 6 h. The mixture was then cooled and kept overnight at −20 °C. The separated solid was removed by filtration, and the cold filtrate was neutralized with saturated aqueous $NaHCO_3$. The organic layer was washed with 5% aqueous NaOH (100 mL) and water until neutral (4 × 200 mL), dried (anhyd Na_2SO_4), and filtered. The solvent was removed under vacuum, and the crude product was purified

by radial chromatography (2.5−3.5% CH_3OH in CH_2Cl_2) collecting the bright blue band to afford 275 mg (82%) of verdin **5** as a 1:1 mixture of racemic and meso diastereomers: mp 205−207 °C; ^{1}H NMR ($CDCl_3$) δ 1.22 (6H, t, ^3J = 7.6 Hz), 1.83 (6H, s), 2.10 (6H, s), 2.52 (4H, q, ^3J = 7.6 Hz), (2.99, 3.04) (2 × 2H, 2 × m), 3.37 (6H, s), (3.730, 3.734) (2 × 3H, 2 × s, racemic and meso), 3.90 (2H, m), 5.94 (2H, s), (6.75, 6.77) (2 × 0.5H, 2 × s, racemic and meso), 8.10 (2H, br s), 9.12 (1H, very br s) ppm; ^{13}C NMR ($CDCl_3$) δ (8.30, 8.34), (9.64, 9.66), 14.48, 17.82, 28.40, 52.10, (58.44, 58.50), 81.20, 96.24, (114.98, 115.12), 128.32, (129.27, 129.31), (133.81, 133.85), 139.76, 141.72, 146.70, 149.75, 172.43, 172.43 ppm; IR ν 3336, 2965, 2933, 2874, 1747, 1698, 1683, 1623, 1589, 1451, 1437, 1388, 1259, 1202, 1176, 1156, 1121, 1098 cm^{-1}; UV−vis λ_{max} nm (ϵ) ($CHCl_3$) 368 (54 000) 642 (15 400), (CH_3OH) 364 (56 200) 643 (15 000), (($CH_3)_2SO$) 372 (55 300), 643 (16 500). Anal. Calcd for $C_{37}H_{46}N_4O_8$ (674.8): C, 65.86; H, 6.87; N, 8.30. Found: C, 65.80; H, 6.99; N, 8.29.

3,17-Diethyl-8,12-bis[2-methoxycarbonyl-2-(methylthio)ethyl]-2,7,13,18-tetramethyl-21*H*,24*H*-bilin-1,19-dione (6, α,α′-Bis(methylthio)mesobiliverdin-XIIIα Dimethyl Ester). Following the preceding procedure for **5**, dipyrrinone **8** (1 mmol) was converted into 263 mg (74%) of bright blue verdin **6** as a 1:1 mixture of racemic and meso diastereomers: mp 197−201 °C; ^{1}H NMR ($CDCl_3$) δ 1.22 (6H, t, ^3J = 7.7 Hz), 1.83 (6H, s), 2.10 (6H, s), (2.21, 2.25) (2 × 3H, 2 × s, racemic and meso), 2.51 (4H, q, ^3J = 7.7 Hz), 2.93 (1H, dd, ^3J = 5.4 Hz, ^2J = 14.2 Hz), 2.95 (1H, dd, ^3J = 6.8 Hz, ^2J = 14.5 Hz), 3.24 (1H, dd, ^3J = 8.3 Hz, ^2J = 14.5 Hz), 3.27 (1H, dd,^3J = 10.0 Hz, ^2J = 14.2 Hz), 3.45 (1H, dd, ^3J = 6.8, 8.3 Hz), 3.51 (1H, dd, ^3J = 5.4, 10.0 Hz), (3.68, 3.71) (2 × 3H, 2 × s, racemic and meso), 5.93 (2H, s), (6.77, 6.80) (2 × 0.5H, 2 × s, racemic and meso), 8.08 (2H, br s) ppm; ^{13}C NMR ($CDCl_3$) δ 8.30, (9.67, 9.73), (14.28, 14.49), 14.41, 17.83, (26.60, 26.82), (47.87, 48.36), (52.30, 52.34), 96.07, (114.84, 115.00), 128.48, 128.93, (134.97, 135.00), (139.99, 140.03), (141.09, 141.24), 146.69, 149.95, (172.04, 172.07), 172.44 ppm; IR ν 3334, 2965, 2920, 2871, 1732, 1696, 1684, 1625, 1589, 1450, 1435, 1387, 1340, 1304, 1263, 1218, 1156, 1096 cm^{-1}; UV−vis λ_{max} nm (ϵ) ($CHCl_3$) 369 (53 900) 642 (14 600), (CH_3OH) 364 (56 600) 642 (14 500), (($CH_3)_2SO$) 373 (55 200) 641 (16 200). Anal. Calcd for $C_{37}H_{46}N_4O_6S_2$ (706.9): C, 62.86; H, 6.56; N, 7.93. Found: C, 62.78; H, 6.67; N, 7.84.

8,12-Bis(2-carboxy-2-methoxyethyl)-3,17-diethyl-2,7,-13,18-tetramethyl-10*H*,21*H*, 23*H*,24*H*-bilin-1,19-dione (1, α,α′-Dimethoxymesobilirubin-XIIIα). A mixture of 168 mg (0.25 mmol) of verdin dimethyl ester **5**, 75 mg of ascorbic acid, 75 mL of THF−CH_3OH (1:1 by vol), 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 into $CHCl_3$ (3 × 150 mL). The solvent from combined extracts was removed under vacuum, and the residual moisture was coevaporated with dry benzene (2 × 50 mL). The crude solid was used immediately in the next step.

The foregoing crude verdin diacid was dissolved in 30 mL of dry deoxygenated CH_3OH. The solution was treated with sodium borohydride (118 mg, 3.13 mmol) added portionwise over 15 min while purging the solution with Ar. After 10 min stirring, the reaction was quenched with 100 mL of H_2O, cooled to 0 °C, and acidified with 10% HCl until pH ∼3.5. The product was extracted with $CHCl_3$ (3 × 80 mL) and, without washing the solvent, was removed under vacuum. The crude material was purified twice by radial chromatography (2−4% CH_3OH in CH_2Cl_2) and recrystallization ($CHCl_3$−Et_2O) to afford 72 mg (44%) of bright yellow rubin **1** (racemic diastereomer): mp >275 °C dec; ^{1}H NMR (($CD_3)_2SO$) δ 1.08 (6H, t, ^3J = 7.5 Hz), 1.77 (6H, s), 1.99 (6H, s), 2.48 (2H, dd, ^3J = 7.8 Hz, ^2J = 14.3 Hz), 2.49 (4H, q, ^3J = 7.5 Hz), 2.56 (2H, dd, ^3J = 6.1 Hz, ^2J = 14.3 Hz), 3.10 (6H, s), 3.38 (2H, dd, ^3J = 6.1, 7.8 Hz), 3.96 (2H, s), 5.95 (2H, s), 9.80 (2H, s), 10.27 (2H, s), 12.48 (2H, s) ppm; ^{13}C NMR (($CD_3)_2SO$) δ 8.16, 9.52, 14.96, 17.24, 23.80, 27.55, 57.22, 80.60, 97.85, 116.11, 121.99, 122.97, 123.53, 127.86, 131.42, 147.30, 172.04, 173.55 ppm; IR ν 3427, 3272, 2965, 2918, 2820, 1692, 1640, 1620, 1436, 1359, 1265, 1245, 1183, 1111, 1024, 980 cm^{-1}; UV−vis in Table 4. Anal. Calcd for $C_{35}H_{44}N_4O_8$ (648.7): C, 64.80; H, 6.84; N, 8.64. Found: C, 64.56; H, 6.82; N, 8.43.

8,12-Bis[2-carboxy-2-(methylthio)ethyl]-3,17-diethyl-2,7,13,18-tetramethyl-10*H*,21*H*,23*H*,24*H*-bilin-1,19-dione (2, α,α′-Bis(methylthio)mesobilirubin-XIIIα). Following the same procedure as for **1** above, 177 mg (0.25 mmol) verdin **6** was converted into a 143 mg (84%) mixture of diastereomeric rubins *rac*-**2** and *meso*-**2**, which was separated by radial chromatography (0.75−1.5% CH_3OH in CH_2Cl_2). Recrystallization of individual bands (using $CHCl_3$−CH_3OH or $CHCl_3$−Et_2O) afforded 72 mg (85%) of racemic diastereomer (*rac*-**2**) and 29 mg (34%) of the meso diastereomer (*meso*-**2**). The former had mp 304−306 °C dec; ^{1}H NMR (($CD_3)_2SO$) δ 1.08 (6H, t, ^3J = 7.5 Hz), 1.77 (6H, s), 1.99 (6H, s), 2.02 (6H, s), 2.31 (2H, dd, ^3J = 6.6 Hz, ^2J = 14.3 Hz), 2.50 (4H, q, ^3J = 7.5 Hz), 2.70 (2H, dd, ^3J = 9.3 Hz, ^2J = 14.3 Hz), 3.00 (2H, dd, ^3J = 6.6, 9.3 Hz), 4.01 (2H, s), 5.94 (2H, s), 9.79 (2H, s), 10.34 (2H, s), 12.32 (2H, s) ppm; ^{13}C NMR (($CD_3)_2SO$) δ 8.07, 9.45, 13.50, 14.84, 17.16, 24.00, 25.81, 47.24, 97.65, 117.14, 122.03, 123.02, 123.05, 128.03, 130.82, 147.24, 171.97, 172.88 ppm; IR ν 3401, 3247, 2967, 2912, 2872, 1666, 1657, 1633, 1433, 1358, 1253, 1186, 1057, 982, 956 cm^{-1}; UV−vis in Table 4. Anal. Calcd for $C_{35}H_{44}N_4O_6S_2$ (680.9): C, 61.74; H, 6.51; N, 8.23. Found: C, 61.56; H, 6.45; N, 8.21.

The meso diastereomer (*meso*-**2**) had mp 283−286 °C dec; ^{1}H NMR (($CD_3)_2SO$) δ 1.08 (6H, t, ^3J = 7.5 Hz), 1.77 (6H, s), 1.99 (6H, s), 2.02 (6H, s), 2.34 (2H, dd, ^3J = 6.1 Hz, ^2J = 14.3 Hz), 2.50 (4H, q, ^3J = 7.5 Hz), 2.70 (2H, dd, ^3J = 9.5 Hz, ^2J = 14.3 Hz), 2.94 (2H, dd, ^3J = 6.1, 9.5 Hz), (3.92, 4.07) (AB, 2H, ^2J = 16.9 Hz), 5.94 (2H, s), 9.79 (2H, s), 10.35 (2H, s), 12.30 (2H, s) ppm; ^{13}C NMR (($CD_3)_2SO$) δ 8.07, 9.45, 13.42, 14.84, 17.16, 23.95, 25.91, 47.11, 97.63, 117.21, 122.03, 123.01, 123.07, 128.01, 130.95, 147.24, 171.96, 172.87 ppm; IR ν 3425, 3341, 2968, 2919, 2874, 1681, 1635, 1624, 1435, 1353, 1315, 1244, 1173, 1059, 979, 957 cm^{-1}; UV−vis in Table 4. Anal. Calcd for $C_{35}H_{44}N_4O_6S_2$ (680.9): C, 61.74; H, 6.51; N, 8.23. Found: C, 61.49; H, 6.71; N, 8.05.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**, **2**, and **5**−**11** are reported (25 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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