Synthesis and Conformational Analysis of a Chiral Fluorinated Bilirubin Analog Lacking Carboxyl Groups

Stefan E. Boiadjiev and David A. Lightner*

Department of Chemistry, University of Nevada, Reno, Nevada 89557-0020

Received March 2, 1999
Revised June 15, 1999

An optically active analog **1** of etiobilirubin-IVγ with a single fluorine on each of the C(8) and C(12) alkyl groups has been synthesized in order to examine its potential for hydrogen bonding with fluorine. Circular dichroism spectroscopy reveals an unusually strong influence of 2,2,2-trifluoroethanol solvent on diastereo-selection of the *M*-helical conformation of (8^1*S*,12^1*S*)-**1**.

J. Heterocyclic Chem., **36**, 969 (1999).

Introduction.

The yellow pigment of jaundice, bilirubin (Figure 1) is an important and structurally interesting mammalian natural product that is produced copiously in normal human metabolism from hemoglobin and other heme proteins [1-3]. Much effort has been devoted toward understanding the properties and metabolism of bilirubin, with special focus on its unique ability to fold into a conformation where the carboxylic acids are joined by hydrogen bonding to the opposing dipyrrinones [4-6], which are known to be avid hydrogen bonding units [7]. Such hydrogen bonding decreases the polarity of the pigment, rendering it nonexcretable in normal metabolism, except following glucuronidation [3, 8]. Synthetic analogs with propionic acids at C(8) and C(12), such as mesobilirubin-XIIIα and **3** (Figure 1), exhibit properties very similar to that of biliru-

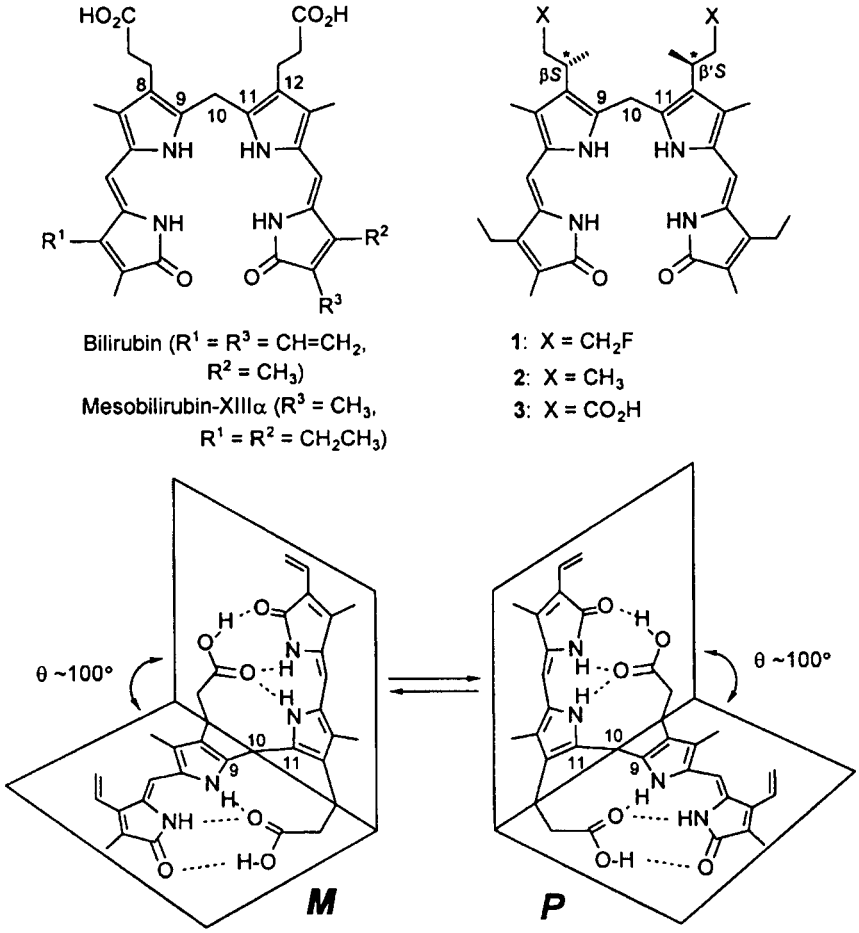


Figure 1. (Upper left) Bilirubin and its analog mesobilirubin-XIIIα in a porphyrin-like conformation. (Upper right) Chrial analogs of mesobilirubin-XIIIα, with stereogenic centers at *. (Lower) Interconverting enantiomeric conformaions of bilirubin, stabilized by intramolecular hydrogen bonds and shaped like ridge tiles. Hydrogen bonds are shown by dashed lines.

bin. Whereas, analogs with propionic acids displaced from their natural locations at C(8) and C(12) cannot engage in conformation stabilizing intramolecular hydrogen bonding and thus have rather different properties from bilirubin.

In recent studies, it was shown that with stereogenic centers at the α or β positions of the propionic acid chains of mesobilirubin-XIIIα, the choice of *R/S* stereochemistry acts as a stereochemical lever to displace the equilibrium between enantiomeric conformations shown in Figure 1 (lower) toward either the ***M*** or ***P*** helicity [9]. Even when the bilirubin pigment has only one propionic acid [10] or is esterified, or when the carboxylic acid group is replaced by methyl (as in **2**) so that intramolecular hydrogen bonding becomes diminished or impossible, β*S*-methyls still act as a molecular gear to direct the conformational stereochemistry toward an *M*-helical pigment, as detected by circular dichroism spectroscopy [11].

Examples of hydrogen bonding between fluorine and hydroxyl or amine hydrogen (C-F···H-X) [12,13] has been searched for among X-ray crystallographic data (Cambridge Structural Database System) and shown to be rare [12]. Calculations *ab initio* indicate that the C(sp^3)-F···H-O hydrogen bond strength is about one-half that of a C-O···H-O hydrogen bond. Given the sensitivity of circular dichroism to structure [9], using circular dichroism to detect conformational differences between **1** and **2** seemed like a potentially beneficial way to explore the possibility that **1** might exhibit special conformational stabilization (beyond that of **2**) due to intramolecular fluorine hydrogen bonds (Figure 2). Accordingly **1** was synthesized as outlined in the following.

Results and Discussion.

Synthesis.

The preparation of **1** was designed along traditional linear tetrapyrrole synthesis modalities that building dipyrroles from monopyrroles and couple two dipyrroles to build tetrapyrroles (Scheme 1) [14]. Fluorine was introduced in mid-synthesis, and the starting material was the known, optically active monopyrrole acid **9** (~100% enantiomeric excess) [9b]. Selective reduction of the carboxylic acid group of **9** using borane-tetrahydrofuran afforded the corresponding primary alcohol in 91% yield, and the alcohol was converted to tosylate **8** [7a] in 79% yield using *p*-toluenesulfonyl chloride in the presence of triethyl amine. Displacement of the tosylate by fluoride was accomplished in 29% yield using potassium fluoride in dimethyl sulfoxide to afford **7**. The lower than expected yield of primary fluoride was due to the formation of aldehyde **6** (in 34% yield) — presumably a product of Kornblum oxidation reaction [15,16] where fluoride ion acts as base (instead of bicarbonate as in [15b]) rather than as nucleophile. This particular side product **6** has potential use in preparing other bilirubin analogs.

Hydrolysis of **7** to its acid, followed by decarboxylation and coupling *in situ* with 5-bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole [9b,c,11,14] in refluxing methanol smoothly afforded bright yellow dipyrrinone **5** in 59% yield. Oxidative self-coupling of **5** using *p*-chloranil afforded the intense blue verdin **4** in 67% yield, and reduction of **4** with sodium borohydride led smoothly to the desired bright yellow rubin **1** in 80% isolated yield.

It is interesting to note that the retention time for **1** is very short on reverse-phase high performance liquid chromatography relative to its non-fluorinated analog **2**: 6.8 minutes for **1** vs. 14.9 minutes for **2** (Figure 1). Both move more rapidly than either bilirubin (17.0 minutes) or mesobilirubin-XIIIα (18.1 minutes), suggesting that they are more polar. The added fluorine (of **1**) apparently makes **1** much more polar, which is peculiar.

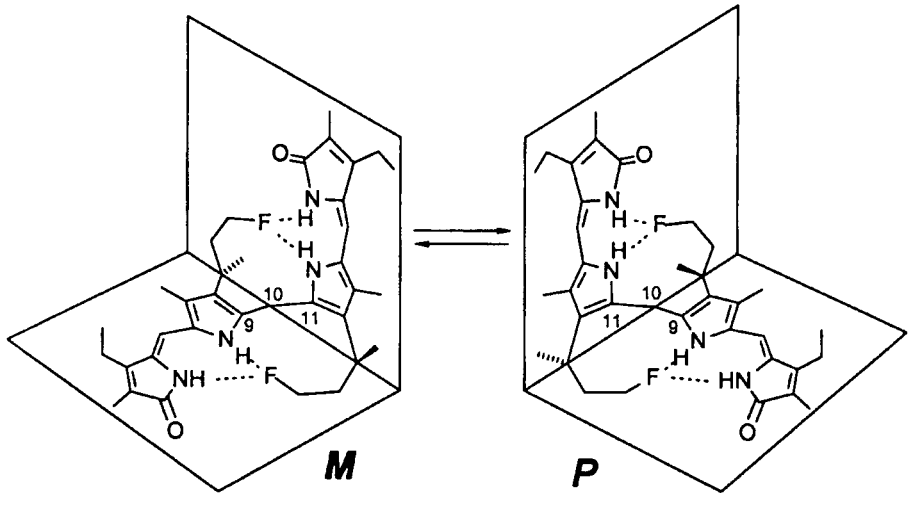
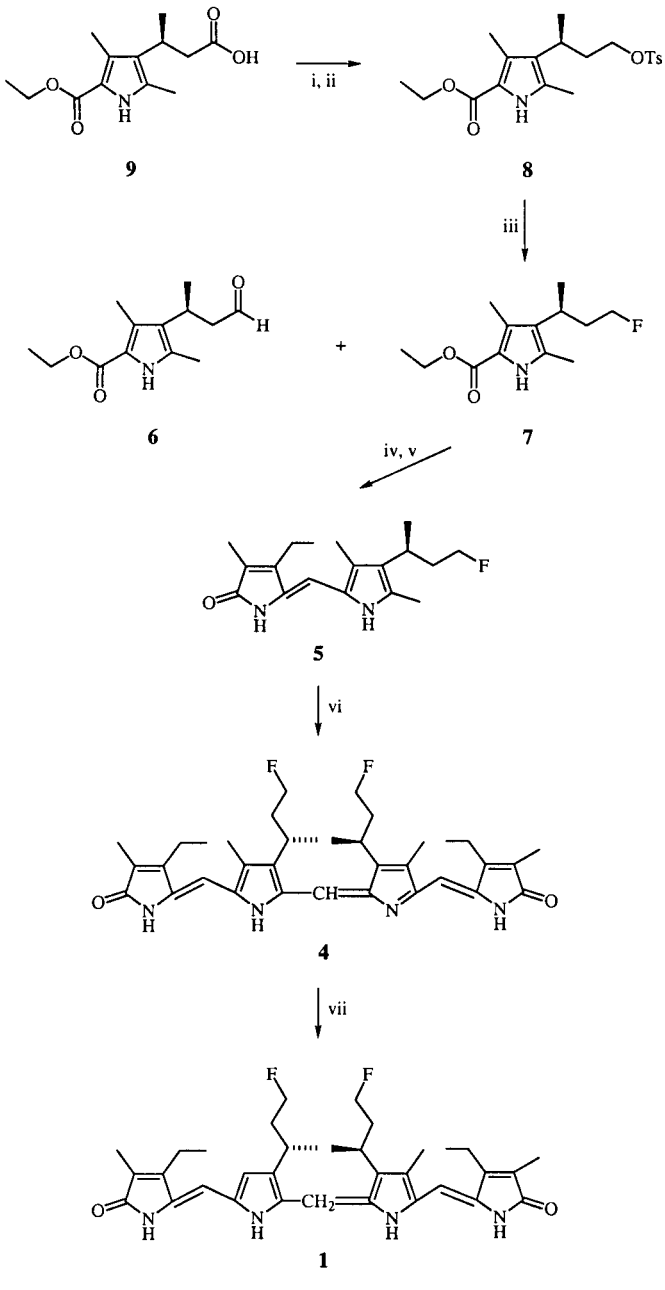


Figure 2. Interconverting, diastereomeric ridge-tile conformations of fluorinated rubin **1** showing C–F···H–N intramolecular hydrogen bonding by dashed lines.

Scheme 1 [a]



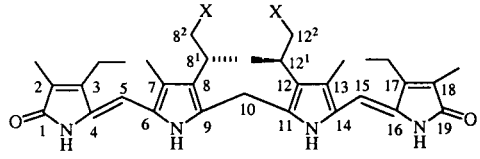
[a] Reagents and conditions: i, borane-tetrahydrofuran; ii, *p*-toluenesulfonyl chloride, triethyl amine; iii, potassium fluoride, dimethyl sulfoxide; iv, sodium hydroxide/water, then nitric acid; v, 5-bromomethylene-4-ethyl- 3-methyl-2-oxo-1*H*-pyrrole, methanol at reflux; vi, *p*-chloranil, formic acid; vii, sodium borohydride, then hydrochloric acid.

Structure and NMR.

The carbon-13 nuclear magnetic resonance (^{13}C-nmr) chemical shifts of the **1** are very similar to that of **2**, and even **3**, and consistent with its constitutional structure. Major differences between **1** and **2** occur in the alkyl chains located at C(8) and C(12). As expected, the carbon signals of these chains in **1** are split from the spin-spin coupling with a single fluorine in each chain. Less noticeable are the relatively greater (~1 ppm) shieldings at C(9) and C(11) and deshielding at C(10) of **1** and **2** compared with **3**. These differences may be diagnostic for intramolecular hydrogen bonding (Figure 1) of the type unique to **3** for nonpolar solvents such as chloroform [9b,10].

Table 1

Comparison of ^{13}C-nmr Chemical Shifts for Mesobilirubin-XIIIα Analogs **1-3** in 10^{-2} *M* Chloroform-d Solutions at 25°C



Position	**1** X = CH_2F	**2** X = CH_3	**3** X = CO_2H
1,19 CONH	174.2	174.1	174.9
2,18 C=	124.3	124.1	123.4
2,18 CH_3	7.5	7.5	7.9
3.17 C=	146.9	146.7	148.4
3^1,17^1 CH_2	17.7	17.8	17.3
3^2,17^2 CH_3	14.7	14.7	14.9
4,16 C=	129.0	128.6	128.3
5,15 CH=	99.8	100	100.3
6,14 C=	122.7	123.3	123.3
7,13 C=	121.8	122.1	122.4
7,13 CH_3	11.0	11.1	11.0
8,12 C=	124.3	124.2	124.8
8^1,12^1 CH	26.9[a]	33.1	26.5
8^1,12^1 CH_3	20.8	20.7	20.9
8^2,12^2 CH_2	36.9[b]	29.6	39.6
8^3,12^3 X	83.0[c]	12.9	180.1
9,11 C=	130.6	131.0	132.9
10 CH_2	22.9	23.2	21.8

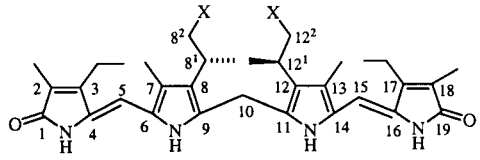
[a] $^3J_{F\text{-}C}$ = 5.9 Hz; [b] $^2J_{F\text{-}C}$ = 19.0 Hz; [c] $^1J_{F\text{-}C}$ = 163.2 Hz.

Consistent with the ^{13}C-nmr results, the relevant ^{1}H-nmr chemical shifts in chloroform-d show great similarity (Table 2). As expected, significant differences are found in the chemical shifts of resonances in the C(8) and C(12) alkyl chains of **1-3**. Aside from these, it may be seen that: (1) the pyrrole NH resonance of **3** is more shielded than the pyrrole NH resonances in **1** and **2**, which have similar chemical shifts; and (2) the C(2) and C(18) methyls of **3** are more deshielded than those in **1** or **2**. The strongly shielded pyrrole NH resonance has been taken as diagnostic of a ridge-tile shape stabilized by intramolecular hydrogen-bonding [9b,c,10,17]. Intramolecular hydrogen bonding is unavailable in **2**, but partial intramolecular hydrogen bonding (ester carbonyl oxygen to pyrrole and lactam NHs) is thought to be a conformation stabilizing factor in the dimethyl ester of **3** [18]. Given the very similar NH chemical shifts in **1** and **2**, the ^{1}H-nmr data indicate that in chloroform solvent,

hydrogen bonding between fluorine and the NHs may be very weak.

Table 2

^{1}H-nmr Assignments for Mesobilirubin-XIIIα Analogs **1-3** in 5 x 10^{-3} *M* Chloroform-d Solutions at 25°



Proton	**1** X = CH_2F	**2** X = CH_3	**3** X = CO_2H
21,24 NHCO	10.73	10.77	10.68
22,23 NH	10.19	10.17	9.04
8^2,12^2 X	4.36[a]	0.83[f]	13.64
2,18 CH_3	1.41	1.40	1.85
3,17 CH_2CH_3	2.32[b]	2.32[b]	2.48[b]
3,17 CH_2CH_3	0.99[c]	0.99[c]	1.11[c]
5,15 CH=	5.91	5.91	6.04
7,13 CH_3	2.15	2.15	2.24
8^1,12^1 CH	3.14[d]	2.81[d]	3.45[d]
8^1,12^1 CH_3	1.35[e]	1.30[e]	1.35[g]
8^2,12^2 CH_2	2.05[d]	1.67[d]	2.70[h],3.08[i]
10 CH_2	4.12	4.12	4.06

[a] 3 x m; [b] q, J = 7.6 Hz; [c] t, J = 7.6 Hz; [d] m; [e] d, J = 7.1 Hz; [f] t, J = 7.3 Hz; [g] d, J = 7.4 Hz; [h] *AB*X, ^{3}J = 3.0, ^{2}J = 18.2 Hz; [i] A*BX*, ^{3}J = 12.3, ^{2}J = 18.2 Hz.

Additional evidence on the solution conformation of **1** comes from high quality transient ^{1}H{^{1}H}-nuclear Overhauser effects measured in chloroform-d applying double pulsed field gradient method [19] (Figure 3). A *syn-Z* configuration is favored in the dipyrrinones, as shown by the strong nuclear Overhauser effects between the lactam and pyrrole NHs, and between the 5/15 =CHs and the 7/13 CH_3s / 3/17-CH_2CH_3s. Strong nuclear Overhauser effects between the 8^1/12^1 CHs and the 10-CH_2 group, and between the 8^1/12^1 CH_3s and the 7/13 CH_3s indicate a preference for the ***M***-helical conformer of **1** (Figure 4). However, weak nuclear Overhauser effects between the 8^1/12^1 CHs and the 7/13 CH_3s, and between the 8^1/12^1 CH_3s and the 10-CH_2 group suggest the possible presence of small amounts of the ***P***-helical conformer, too. On the basis of the relative strength of the nuclear Overhauser effects, one could say that the ***M***-helicity is favored over the ***P***, but not to the exclusion of the latter. The conclusions from 1D nuclear Overhauser effect spectra were confirmed by a 2D-ROESY [20] experiment, Figure 4.

Interestingly, weak nuclear Overhauser effects were also detected between the *exo* methyls at C(2) and C(18) and the pyrrole NHs of **1** in chloroform-d. Weak nuclear Overhauser effects were found also between these methyls and the 10-CH_2 group — data which suggest the presence of dimers at the concentrations used (~5 x 10^{-3} *M*). Similar intermolecular nuclear Overhauser effects have been seen in dipyrrinones related to **5**, where dipyrrinone to dipyrrinone hydrogen bonding prevails [7c].

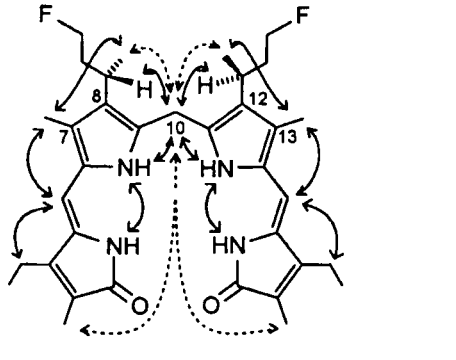


Figure 3. ^{1}H{^{1}H}-Nuclear Overhauser effects seen in chloroform-d solutions of **1**. Solid arrows indicate stong nuclear Overhauser effects; dashed arrows indicate medium nuclear Overhauser effects. Very weak nuclear Overhauser effects are seen between the 8^1/12^1-CH_3 groups and the 10-CH_2, and between the 8^1/12^1 CH and the 7/13 CH_3s. A weak nuclear Overhauser effect is also seen between the 2/18 CH_3s and the 10-CH_2 and pyrrole NHs.

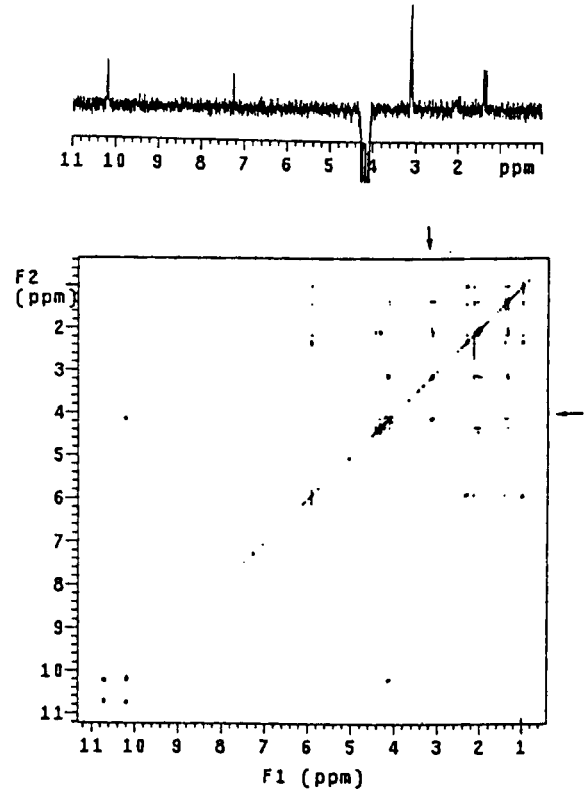


Figure 4. (Upper) Transient ^{1}H{^{1}H}-nuclear Overhauser effect spectrum of **1** in chloroform-d. Irradiated 10-CH_2 group which showed strong nuclear Overhauser effect on 8^1/12^1 CHs and weak nuclear Overhauser effect on 8^1/12^1 CH_3s as well as on pyrrole NHs and 2/18 CH_3s. (Lower) ROESY spectrum of **1** in chloroform-d. The arrows point to the off-diagonal peak due to nuclear Overhauser effect between 10-CH_2 and 8^1/12^1 CHs.

Intermolecular hydrogen bonding in bilirubin dimethyl ester is believed to explain the presence of dimers [6a], thought to be prevalent in chloroform solutions at high concentrations (10^{-2} to 10^{-3} *M*) on the basis of vapor pressure osmometry studies [2]. More recently Ribó *et al.* [21] have used a similar technique to show the presence of dimers (Φ (MW_{calc}/MW_{exp}) in mesobilirubin-IXα dimethyl ester, $\Phi = 0.65$ (2 - 5 x 10^{-2} *M*) and mesobilirubin-XIIIα dimethyl ester, $\Phi = 0.50$ (2.5 x 10^{-2} *M*). One might thus assume the presence of dimers in **1** (and **2**).

Conformation and Circular Dichroism.

Circular dichroism spectroscopy offers a potentially more sensitive way to elicit conformational information and detect interactions with the fluorines of **1**. Moderately strong to weak bisignate circular dichroism curves are

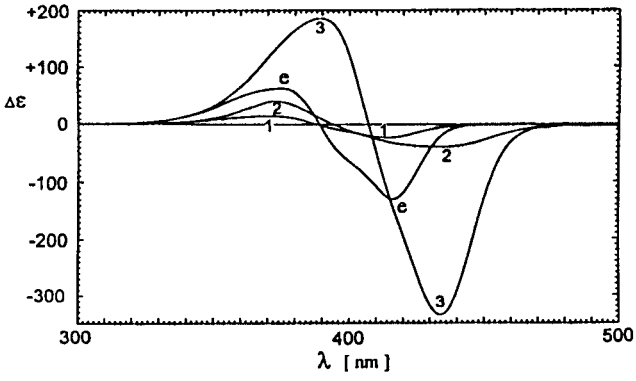


Figure 5. Circular dichroism spectra of 10^{-5} *M* **1**, **2**, **3**, and **e** (the dimethyl ester of **3**) in chloroform with compound numbers assigned to each curve.

Table 3

Solvent Dependence of the Crcular Dichroism Spectra of Pigments **1-3** [a]

Pigment	Solvent	∈ [b]	Circular dichroism Δ∈ (max) at λ(1)	Circular dichroism λ at Δ∈ = 0	Circular dichroism Δ∈ (max) at λ(2)	UV-Visible ∈ (max)	UV-Visible λ (nm)
1	Benzene	2.3	+ 10 (420)	386	- 7 (371)	68,300	378
2			- 45 (440)	394	+ 43 (374)	67,700	380
3			- 362 (434)	406	+191 (390)	60,000	432
1	Chloroform	4.7	- 24 (414)	389	+ 15 (371)	62,800	377
2			- 40 (433)	394	+ 42 (373)	64,700	379
3			- 337 (434)	407	+ 186 (389)	55,800	431
1	Tetrahydrofuran	7.3	- 4 (435)	416	+ 5 (378)	67,600	375
2			- 35 (428)	393	+ 40 (371)	64,200	378
3			- 338 (433)	406	+ 188 (390)	57,900	431
1	Dichloromethane	8.9	- 20 (417)	389	+ 19 (369)	71,900	375
2			- 46 (431)	393	+ 50 (373)	67,500	378
3			- 319 (433)	407	+180 (392)	56,400	430
1	Acetone	20.7	- 15 (429)	390	+ 17 (369)	68,400	373
2			- 41 (425)	392	+ 45 (370)	64,000	376
3			- 322 (430)	404	+182 (387)	57,100	427
1	Ethanol	24.3	+ 7 (412)	375	- 2 (361)	50,600	392
2			- 21 (434)	403	+ 18 (388)	47,300	394
3			- 284 (434)	405	+ 168 (389)	57,600	426
1	2,2,2-Trifluoroethanol	26.5	- 76 (421)	396	+ 48 (376)	46,700	404
2			- 13 (440)	409	+ 11 (375)	45,200	394
3			-292 (432)	406	+187 (386)	55,200	427
1	Methanol	32.6	+ 5 (412)	371	- 2 (352)	50,100	393
2			- 20 (430)	403	+ 17 (382)	47,600	395
3			- 285 (431)	405	+177 (386)	60,800	425
1	Acetonitrile	36.2	- 23 (424)	387	+ 23 (366)	71,500	372
2			- 38 (429)	392	+ 42 (370)	66,900	375
3			- 315 (429)	403	+ 181 (384)	56,700	423
1	*N,N*-Dimethylformamide	36.7	- 9 (418)	391	+ 10 (376)	54,000	418
2			- 24 (425)	395	+ 18 (376)	50,300	423
3			- 246 (429)	404	+165 (386)	54,000	421
1	Dimethylsulfoxide	46.5	- 14 (422)	396	+ 13 (380)	59,600	425
2			- 30 (428)	398	+ 20 (386)	55,400	430
3			+ 23 (425)	385	- 6 (369)	56,700	425
1	*N*-Methylformamide	182	- 4 (425)	405	+ 7 (380)	50,300	421
2			- 21 (432)	403	+ 17 (387)	49,500	393
3			- 359 (427)	400	+200 (383)	66,000	426

[a] All solutions are ~1.5 x 10^{-5} *M* and contain 2% v/v chloroform; measurements at 23°. [b] Dielectric constant from A. J. Gordon and R. A. Ford, The Chemist's Companion, Wiley, NY, 1972, pp 4-8.

found (Table 3) and are characteristic of a molecular exciton system [4,22,23] in which the two dipyrrinone chromophores interact by coupling locally excited $\pi \rightarrow \pi^*$ transitions (electric transition dipole coupling). Dipyrrinone chromophores have strongly allowed long-wavelength electronic transitions [4,11,23,24] and may interact through resonance splitting, *i.e.*, by electrostatic interaction of the local transition moment dipole oriented along the long axis of each dipyrrinone. Such intramolecular exciton splitting interaction produces two long wavelength transitions in the ultraviolet-visible spectrum and two corresponding bands in the circular dichroism spectrum. One band is higher in energy, and one is lower, with the magnitude of splitting being dependent on the strength and relative orientation of the electric dipole transition moment from each dipyrrinone. According to exciton chirality theory [22], a long wavelength negative, short wavelength positive circular dichroism Cotton effects indicate a negative exciton chirality and a negative helical disposition of the dipyrrinone long axis polarized transition moments. Usually this corresponds to the *M*-helical enantiomeric type of Figures 1 and 2 [9,23], but, as has been shown earlier, opening the interplanar angle of either enantiomer without inverting molecular chirality can cause the relative orientation of the relevant dipyrrinone transition moments to invert helicity, thus inverting the signed order of the circular dichroism couplet [25].

The effectiveness of the 8^1 and 12^1 methyls of **1-3** in displacing the $M \leftrightarrows P$ conformational equilibrium may be seen in their circular dichroism spectra (Figure 5). This is especially noticeable in the rubin acid **3**, where intramolecular hydrogen bonding stabilizes the ridge-tile conformation and brings into play nonbonded steric interactions between its 8^1S, 12^1S methyls and the C(10) methylene group that destabilize the *P* helical conformation and favor the *M*. For this reason negative exciton chirality circular dichroism spectra are seen for **3** in a variety of solvents (Table 3) that do not strongly perturb the intramolecular hydrogen bonding. In dimethyl sulfoxide, solvent intercalation into the hydrogen bond matrix lessens the conformational preference dictated by the 8^1, 12^1 methyls, and the circular dichroism intensity shrinks considerably.

Even when intramolecular hydrogen bonding is impossible, as when the propionic acids of **3** are replaced with 2-butyl chains, moderately strong bisignate circular dichroism curves are still seen (Figure 6). The signs remain the same as those of **3**, over a wide range of solvent polarity, except in the special case of dimethyl sulfoxide solvent, and the magnitudes show no great variation (Table 3). In strong contrast to the parent hydrocarbon **2**, the circular dichroism spectra of **1** show considerable variation, from a weakly negative exciton chirality (tetrahydrofuran, dimethylformamide), moderately negative (chloroform, dichloromethane, acetone, acetonitrile, dimethyl sulfoxide) to weakly positive (benzene, ethanol, methanol). Within each grouping, one finds a wide range of solvent dielectric, suggesting that the observed effects are unrelated to solvent polarity and protic *vs* aprotic nature. Unusual among the solvents, and unlike its influence on **2** and **3**, 2,2,2-trifluoroethanol provides the most intense circular dichroism observed for **1**. In fact, the negative chirality circular dichroism seen for **1** is stronger than any circular dichroism observed for the parent **2** (Table 3). Curiously, while the change from ethanol to 2,2,2-trifluoroethanol solvent scarcely changes the circular dichroism spectra of **2** and **3**, it has a very profound effect on **1** (Figure 6). At present, it is unclear why the fluorinated solvent should have such a large effect on the circular dichroism of **1**.

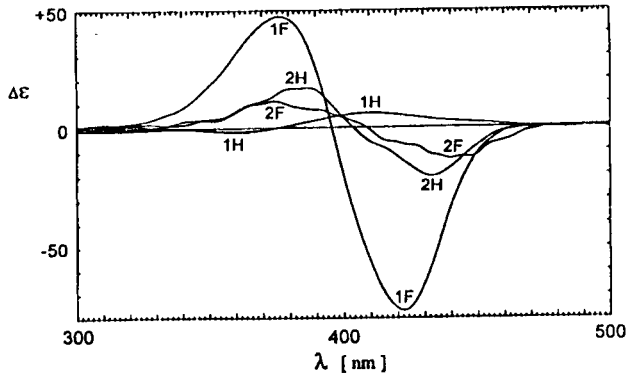


Figure 6. Circular dichroism spectra of 10^{-5} *M* **1** and **2** in 2,2,2-trifluoroethanol (1F and 2F, respectively) and in ethanol (1H and 2H, respectively). The compound numbers are assigned on each curve.

The circular dichroism data suggest a special effect of fluorine (in **1**) not seen in the parent **2**. One is tempted to think that the carbon-fluorine dipole is responsible. Whether this includes fluorine to dipyrrinone NH hydrogen bonding, such as that in Figure 2, is uncertain on the basis of the evidence gathered.

Conformation from Molecular Dynamics Calculations.

Previously we showed the insight that molecular modelling calculations can provide to problems of bile pigment conformational analysis [4,9b,11,17]. Computations of the minimum energy conformations (Figure 7) of **1** and **2**, using the Sybyl force field [4] indicate that *M*-helical ridge-tile conformations (see Figure 1) lie at the global minimum. The various torsion (Φ) and dihedral (θ) angles that define bilirubin conformations are shown in Table 4 and indicate that while the *non-hydrogen bonded* global minimum conformations of **1** and **2** are rather similar, as might be expected, they do not differ greatly from the intramolecularly hydrogen bonded global minimum con-

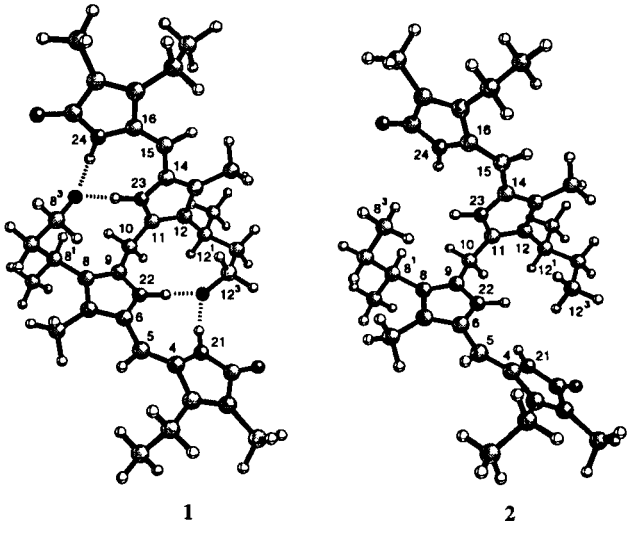
formations of **1** and **3**. All adopt an *M*-helical, ridge-tile shape, and the dipyrrinones are more planar where intramolecular hydrogen bonding is present. Unlike bilirubin, conformation-stabilizing intramolecular hydrogen bonding is impossible in **2**, but the (β*S*,β'*S*) methyls appear to act like molecular steric ratchets in directing the conformation toward the more stable *M*-helical conformer. In **1**, the *M*-helical conformation is also the more stable, and these findings are largely consistent with those from the circular dichroism analysis (above). For **1**, however, one might envision the possibility of some stabilization coming from N-H···F-CH_2- intramolecular hydrogen bonds. In fact, comparison of **1** in its hydrogen bonded and non-hydrogen bonded global minima indicates a stabilization of ~13.1 kcal/mole in the former, in its non-solvated state. Although the number may seem large, the data are for unsolvated rubins, and the same computation on **3** predicts that the global minimum is ~28.5 kcal/mole more stable than its non-hydrogen bonded global minimum. What sort of effects solvation might play on possible hydrogen bonding is unclear and is currently under study.

Table 4

Torsion and Dihedral Angles in Global Minimum Conformations Determined by Molecular Dynamics Computations [a]

Torsion Angles (Φ°) and Dihedral Angles (θ°)	Compound 1 H-bonded	Compound 1 Non-H-bonded	Compound 2	Compound 3
Φ (6-5=4-21)	- 1.4	- 3.3	- 3.6	+ 1.1
Φ (14-15=16-24)	- 1.4	- 3.5	- 3.6	+ 1.2
Φ (4=5-6-22)	- 8.5	- 33.7	- 32.3	+ 17.0
Φ (16=15-14-23)	- 9.4	- 33.7	- 32.3	+ 17.2
Φ (11-10-9-22)	- 65.6	- 70.9	- 61.4	- 64.0
Φ (9-10-11-23)	- 65.5	- 70.9	- 61.3	- 64.3
θ (pyrroles)	- 93.2	- 100.0	- 87.3	- 87.0
θ (dipyrrinones)	- 89.6	- 67.9	- 67.0	- 88.3

[a] Using Sybyl version 6.4 on an SGI Octane/SSE workstation. See also ref. 4.



1 2

Figure 7. Ball and stick representations for the global minimum energy conformations of **1** and **2**.

EXPERIMENTAL

General Procedures.

All circular dichroism spectra were recorded on a JASCO J-600 instrument, and ultraviolet-visible spectra were recorded on a Perkin-Elmer Lambda 12 or Cary 219 spectrophotometer. Nuclear magnetic resonance (nmr) spectra were obtained on a GN-300 or a Varian Unity Plus spectrometer operating at 300 MHz and 500 MHz (for ^{1}H-nmr), respectively. Chloroform-d solvent (unless otherwise noted) was used, and chemical shifts were reported in δ ppm referenced to the residual chloroform ^{1}H signal at 7.26 ppm and ^{13}C signal at 77.00 ppm. J-modulated spin-echo experiment (*A*ttached *P*roton *T*est) was used to obtain and assign ^{13}C-nmr spectra. Optical rotations were measured on a Perkin Elmer model 141 polarimeter. High performance liquid chromatographic analyses were carried out on a Perkin Elmer Series 410 high-pressure liquid chromatograph with a Perkin Elmer LC-95 ultraviolet-visible spectrophotometric detector (set at 420 nm) equipped with a Beckman-Altex ultrasphere IP 5 μ C-18 ods column (25 x 0.46 cm) kept at 34°. The flow rate was 1.0 ml per minute, and the mobile phase was 0.1 *M* di-*n*-octylamine acetate buffer in 5% aqueous methanol (*p*H 7.7 at 22°). Radial chromatography was carried out on Merck silica gel PF_{254} with calcium sulfate binder preparative layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA) with 1, 2, or 4 mm rotors. Melting points were determined on a Mel-Temp capillary apparatus and are uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ.

Commercial reagents were used as received from Aldrich or Acros. The spectral data were obtained in spectral grade solvents (Aldrich or Fisher). High performance liquid chromatography grade solvents (Fisher) were dried according to standard procedures [26] and distilled prior to use.

(+)-(*S*)-3-(2,4-Dimethyl-5-ethoxycarbonyl-1*H*-pyrrol-3-yl)butanoic Acid (**9**).

This compound was synthesized as racemate and resolved into enantiomers *via* 1:1 salt with brucine as described earlier [9b]. It had mp 148-150° and $[\alpha]_D^{20}$ +30.5° (*c* 0.8, ethanol).

(+)-(*S*)-3-(2,4-Dimethyl-5-ethoxycarbonyl-1*H*-pyrrol-3-yl)butyl *p*-Toluenesulfonate (**8**).

This compound was obtained from the corresponding alcohol as previously described [7a]. It had mp 85-87° and $[\alpha]_D^{20}$ +12.6° (*c* 1.0, ethanol).

(+)-(*S*)-Ethyl 3,5-Dimethyl-4-(3-fluoro-1-methylpropyl)pyrrole-2-carboxylate (**7**).

A mixture of 16.7 g (42.5 mmoles) of tosylate **8**, 40.0 g (425 mmoles) of finely ground potassium fluoride dihydrate, and 90 ml of purified dimethyl sulfoxide was heated on an oil bath at 190° for 2.5 hours. After cooling to 90° water was added drop-

wise (200 ml) and the mixture was stirred at 0° for 2 hours. The precipitate was filtered, washed with water and dried. The crude product was separated by column chromatography on silica gel (5-20% acetone in hexane) to afford after recrystallization from ethyl acetate/hexane: (a) 2.99 g (29%) of fluoride **7**, (b) 3.40 g (34%) of aldehyde **6**, and 2.75 g (27%) of (+)-alcohol corresponding to starting tosylate **8**.

(a) Fluoride **7** had mp 82-83°; $[\alpha]_D^{20}$ +31.5°(*c* 1.0, ethanol); ^{1}H-nmr: δ 1.28 (3H, d, J = 7.2 Hz), 1.34 (3H, t, J = 7.1 Hz), 1.95 (2H, m), 2.23 (3H, s), 2.31 (3H, s), 2.95 (1H, m), 4.29 (2H, q, J = 7.1 Hz), 4.31 (2H, 3 x m), 8.61 (1H, br.s) ppm; ^{13}C-nmr: δ 11.1, 12.3, 14.5, 20.5, 26.3 (d, $^3J_{C\text{-}F}$ = 4.9 Hz), 36.81 (d, $^2J_{C\text{-}F}$ = 19.2 Hz), 59.6, 82.7 (d, $^1J_{C\text{-}F}$ = 163.1 Hz), 116.9, 124.1, 126.7, 129.5, 161.9 ppm; ms: m/z (relative intensity) 241 (33), 194 (83), 180 (19), 148 (100) amu.

Anal. Calcd. for $C_{13}H_{20}FNO_2$ (241.3): C, 64.70; H, 8.35; N, 5.80; F, 7.87. Found: C, 64.95; H, 8.49; N, 5.52; F, 7.56

(b) Aldehyde **6** had mp 85-86°; $[\alpha]_D^{20}$ +20.2°(*c* 1.1, ethanol); ^{1}H-nmr: δ 1.27 (3H, d, J = 7.2 Hz), 1.33 (3H, t, J = 7.1 Hz), 2.26 (3H, s), 2.34 (3H, s), 2.71 (2H, *AB*X, J = 7.4, 9.1, 15.9 Hz), 3.37 (1H, m), 4.28 (2H, q, J = 7.1 Hz), 8.85 (1H, br.s), 9.66 (1H, t, J = 2.1 Hz) ppm; ^{13}C-nmr: δ 11.1, 12.6, 14.5, 20.7, 25.2, 50.1, 59.7, 117.0, 124.0, 126.2, 128.9, 161.6, 202.3 ppm.

(+)-(*S*)-4-Ethyl-9-(3-fluoro-1-methylpropyl)-3,8,10-trimethyl-2-oxo-1,11-dihydrodipyrrinone (**5**).

A mixture of 2.41 g (10 mmoles) of pyrrole ester **7**, 2.00 g (50 mmoles) of sodium hydroxide, 8 ml of 50% aqueous sodium nitrate, and 20 ml of ethanol was heated at reflux for 3 hours. The ethanol was evaporated under vacuum, and the residue was carefully acidified at -15° with a mixture of 50% aqueous sodium nitrate : concentrated nitric acid = 5:1. The pyrrole α-acid formed as colorless precipitate was filtered, washed with 2 x 5 ml of water, dried under vacuum (phosphorous pentoxide) for 3 hours and used in the next step without further characterization.

The foregoing pyrrole α-acid was dissolved in 35 ml of dry methanol containing 2.16 g (10.0 mmoles) of 5-bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole [27] and the mixture was heated at reflux for 6 hours. After chilling overnight at -25° the precipitated product was filtered and washed with cold methanol. Recrystallization from chloroform/methanol afforded 1.81 g (59%) of bright yellow dipyrrinone **5**. It had mp 209-211°; $[\alpha]_D^{20}$ +79.0° (*c* 0.6, chloroform); ^{1}H-nmr: δ 1.20 (3H, t, J = 7.5 Hz), 1.33 (3H, d, J = 7.2 Hz), 1.96 (3H, s), 2.03 (2H, m), 2.18 (3H, s), 2.47 (3H, s), 2.57 (2H, q, J = 7.5 Hz), 3.01 (1H, m), 4.39 (2H, 3 x m), 6.16 (1H, s), 10.39 (1H, br.s), 11.41 (1H, br.s) ppm; ^{13}C-nmr: δ 8.5, 10.3, 12.5, 15.0, 17.9, 20.6, 26.4 (d, $^3J_{C\text{-}F}$ = 4.8 Hz), 37.0 (d, $^2J_{C\text{-}F}$ = 19.4 Hz), 82.9 (d, $^1J_{C\text{-}F}$ = 163.5 Hz), 100.9, 122.3, 122.4, 123.2, 124.3, 127.0, 131.2, 148.3, 174.1 ppm.

Anal. Calcd. for $C_{18}H_{25}FN_2O$ (304.4): C, 71.02; H, 8.28; N, 9.20; F, 6.24. Found: C, 70.58; H, 8.41; N, 8.97; F, 5.80.

(-)-(1*S*,1'*S*)-3,17-Diethyl-8,12-bis(3-fluoro-1-methylpropyl)-2,7,13,18-tetramethyl-(21*H*,24*H*)-bilin-1,19-dione (**4**).

A mixture of 457 mg (1.50 mmoles) of dipyrrinone **5**, 922 mg (3.75 mmoles) of *p*-chloranil, 350 ml of dichloromethane, and 15 ml of formic acid was heated at reflux for 24 hours. The mixture volume was reduced by distillation to one half and reflux was continued more 12 hours. Then the mixture was chilled overnight at -20°. The separated solid was filtered and washed with cold dichloromethane. The blue cold filtrate was neutralized with 5% aqueous sodium bicarbonate, the organic layer was washed with 2 x 100 ml of 4% sodium hydroxide, 4 x 100 ml of water, dried (anhydrous sodium sulfate), filtered, and the solvent was removed under vacuum. The residue was purified by radial chromatography on silica gel (eluent 1-3% methanol in dichloromethane) to afford 297 mg (67%) of bright blue mesobiliverdin analog **4**. It had mp 221-224°; $[\alpha]_{436}^{20}$ -280° (*c* 2.9 x 10^{-3}, chloroform); ^{1}H-nmr: δ 1.23 (6H, t, J = 7.6 Hz), 1.41 (6H, d, J = 7.2 Hz), 1.83 (6H, s), 2.07 (4H, m), 2.13 (6H, s), 2.53 (4H, q, J = 7.6 Hz), 3.21 (2H, m), 4.36 (4H, 3 x m), 5.99 (2H, s), 6.92 (1H, s), 7.99 (2H, br.s) ppm; ^{13}C-nmr: δ 8.2, 10.2, 14.4, 17.8, 21.1, 26.9 (d, $^3J_{C\text{-}F}$ = 3.9 Hz), 37.6 (d, $^2J_{C\text{-}F}$ = 19.4 Hz), 82.2 (d, $^1J_{C\text{-}F}$ = 164.4 Hz), 96.05, 115.1, 127.3, 128.1, 139.6, 140.5, 141.8, 146.7, 150.0, 172.2 ppm.

Anal. Calcd. for $C_{35}H_{44}F_2N_4O_2$ (590.7): C, 71.16; H, 7.51; N, 9.48; F, 6.43. Found: C, 71.45; H, 7.45; N, 9.19; F, 6.65.

(-)-(1*S*,1'*S*)-3,17-Diethyl-8,12-bis(3-fluoro-1-methylpropyl)-2,7,13,18-tetramethyl-(10*H*,21*H*,23*H*,24*H*)-bilin-1,19-dione (**1**).

To a cooled to 0° solution of 118 mg (0.2 mmole) of verdin **4** in 40 ml of dry deoxygenated tetrahydrofuran under nitrogen was added 756 mg (20 mmoles) of sodium borohydride followed by slow addition of 20 ml of dry methanol. After 30 minutes stirring at 0°, water (150 ml) was added followed by careful acidification with 10% hydrochloric acid. The product was extracted with chloroform (3 x 30 ml), washed with water (3 x 100 ml), dried (anhydrous sodium sulfate), filtered, and the solvent was evaporated under vacuum. After radial chromatography purification (eluent 1.5-3.0% methanol in dichloromethane) and recrystallization from chloroform-methanol, 95 mg (80%) of bright yellow mesobilirubin **1** was obtained. It had mp 264-266° dec; $[\alpha]_D^{20}$ -470 (*c* 2.7 x 10^{-2}, chloroform); ^{1}H-nmr: δ 0.99 (6H, t, J = 7.6 Hz), 1.35 (6H, d, J = 7.1 Hz), 1.41 (6H, s), 2.05 (4H, m), 2.15 (6H, s), 2.32 (4H, q, J = 7.6 Hz), 3.14 (2H, m), 4.12 (2H, s), 4.36 (4H, 3 x m), 5.91 (2H, s), 10.19 (2H, br.s), 10.73 (2H, br.s) ppm; ^{13}C-nmr: δ 7.5, 11.0, 14.7, 17.7, 20.8, 22.9, 26.8 (d, $^3J_{C\text{-}F}$ = 5.9 Hz), 36.9 (d, $^2J_{C\text{-}F}$ = 19.0 Hz), 83.0 (d, $^1J_{C\text{-}F}$ = 163.2 Hz), 99.8, 121.8, 122.7, 123.5, 124.3, 129.0, 130.6, 146.9, 174.2 ppm.

Anal. Calcd. for $C_{35}H_{46}F_2N_4O_2$ (592.7): C, 70.92; H, 7.82; N, 9.45. Found: C, 70.39; H, 7.85; N, 9.14

Acknowledgment.

We thank the U.S. National Institutes of Health (HD 17779) for generous support of this work. Proffessor S.E. Boiadjiev is on leave from the Institute of Organic Chemistry, Bulgarian Academy of Sciences.

REFERENCES AND NOTES

[1] A. F. McDonagh, Bile Pigments: Bilatrienes and 5,15-Biladienes in The Porphyrins, Vol **VI**, D. Dolphin, ed, Academic Press: New York, 1979, Chapter 6.

[2] H. Falk, The Chemistry of Linear Oligopyrroles and Bile Pigments, Springer Verlag: New York, Vienna, 1989.

[3] J. R. Chowdury, A. W. Wolkoff, N. R. Chowdury, I. M. Arias, Hereditary Jaundice and Disorders of Bilirubin Metabolism in The Metabolic and Molecular Bases of Inherited Disease, Vol **II**, C. R. Scriver, A. L. Beaudet, W. S. Sly and D. Valle, eds, McGraw-Hill Inc., New York, 1995, 2161-2208.

[4] R. V. Person, B. R. Peterson and D. A. Lightner, *J. Am. Chem. Soc.*, **116**, 42 (1994).

[5a] R. Bonnett, J. E. Davies, M. B. Hursthouse and G. M. Sheldrick, *Proc. R. Soc. Lond.*, **B202**, 249 (1978); [b] G. LasBas, A. Allegret, Y. Mauguen, C. DeRango and M. Bailly, *Acta Crystallogr., Sect. B*, **B36**, 3007 (1980); [c] W. Becker and W. S. Sheldrick, *Acta Crystallogr., Sect. B*, **B34**, 1298 (1978).

[6a] D. Kaplan and G. Navon, *Israel J. Chem.*, **23**, 177 (1983); [b] D. Kaplan and G. Navon, *Org. Magn. Reson.*, 198 (1983); [c] D. Kaplan and G. Navon, *Biochem. J.*, **201**, 605 (1982).

[7a] S. E. Boiadjiev, D. T. Anstine and D. A. Lightner, *J. Am. Chem. Soc.*, **117**, 8727 (1995); [b] S. E. Boiadjiev, D. T. Anstine, E. Maverick and D. A. Lightner, *Tetrahedron: Asymmetry* **6**, 2253 (1995); [c] D. F. Nogales, J -S. Ma and D. A. Lightner, *Tetrahedron*, **49**, 2361 (1993).

[8] A. F. McDonagh and D. A. Lightner, in Hepatic Metabolism and Disposition of Endo and Xenobiotics, Falk Symposium No. 57, K. W. Bock, W. Gerok and S. Matern, eds, Kluwer, Dordrecht, The Netherland, Chapter 5, p 47 (1991).

[9a] G. Puzicha, Y-M. Pu and D. A. Lightner, *J. Am. Chem. Soc.*, **113**, 3583 (1991); [b] S. E. Boiadjiev, R. V. Person, G. Puzicha, C. Knobler, E. Maverick, K. N. Trueblood and D. A. Lightner, *J. Am. Chem. Soc.*, **114**, 10123 (1992); [c] S. E. Boiadjiev and D. A. Lightner, *Tetrahedron: Asymmetry*, **8**, 2115 (1997).

[10] S. E. Boiadjiev and D. A. Lightner, *Tetrahedron: Asymmetry*, **8**, 3603 (1997).

[11] S. E. Boiadjiev, W. P. Pfeiffer and D. A. Lightner, *Tetrahedron*, **53**, 14547 (1997).

[12] J. K. Howard, V. J. Hoy, D. O'Hagen and G. T. Smith, *Tetrahedron*, **52**, 12613 (1996).

[13a] V. R. Thalladi, H -C. Weiss, D. Bläser, R. Boese, A. Nangia and G. R. Desiraju, *J. Am. Chem. Soc.*, **120**, 8702 (1998); [b] M. Pham, M. Gdaniec and T. Połoński, *J. Org. Chem.*, **63**, 3731 (1998).

[14] S. E. Boiadjiev and D. A. Lightner, *Synlett*, 777 (1994).

[15a] N. Kornblum, J. W. Powers, G. J. Anderson, W. J. Jones, H. O. Larson, O. Levand and W. M. Weaver, *J. Am. Chem. Soc.*, **79**, 6562 (1957); [b] N. Kornblum, W. J. Jones and G. J. Anderson, *J. Am. Chem. Soc.*, **81**, 4113 (1959); [c] H. R. Nace and J. J. Monagle, *J. Org. Chem.*, **24**, 1792 (1959).

[16] Reviews on oxidation with dimethyl sulfoxide: [a] A. J. Mancuso and D. Swern, *Synthesis*, 165 (1981); [b] W. W. Epstein and F. W. Sweat, *Chem. Rev.*, **67**, 247 (1967).

[17a] A. Kar and D. A. Lightner, *Tetrahedron*, **54**, 5151 (1998); [b] A. Kar and D. A. Lightner, *Tetrahedron*, **54**, 12671 (1998).

[18] S. E. Boiadjiev, D. T. Anstine and D. A. Lightner, *Tetrahedron: Asymmetry*, **5**, 1945 (1994).

[19a] J. Stonehouse, P. Adell, J. Keeler and A. J. Shaka, *J. Am. Chem. Soc.*, **116**, 6037 (1994); [b] K. Stott, J. Keeler, Q. N. Van and A. J. Shaka, *J. Magn. Reson.*, **125**, 302 (1997).

[20a] H. Kessler, C. Griesinger, R. Kerssebaum, K. Wagner and R. R. Ernst, *J. Am. Chem. Soc.*, **109**, 607 (1987); [b] A. Bax and D. G. Davis, *J. Magn. Reson.*, **63**, 207 (1985).

[21] J. Crusats, A. Delgado, J-A. Ferrera, F. Rubires and J. M. Ribó, *Monats. Chem.*, **129**, 741 (1998).

[22] N. Harada and K. Nakanishi, Circular Dichroic Spectroscopy - Exciton Coupling in Organic Stereochemistry, University Science Books: Mill Valley, CA (1983).

[23] D. A. Lightner, J. K. Gawroński and W. M. D. Wijekoon, *J. Am. Chem. Soc.*, **109**, 6354 (1987).

[24a] Y. S. Byun and D. A. Lightner, *J. Org. Chem.*, **56**, 6027 (1991); [b] Y. S. Byun and D. A. Lightner, *Tetrahedron*, **47**, 9759 (1991).

[25] Y-M. Pu, A. F. McDonagh and D. A. Lightner, *J. Am. Chem. Soc.*, **115**, 377 (1993).

[26] D. D. Perrin and W. L. F. Armarego, Purification of Laboratory Chemicals, 3rd Ed, Pergamon Press, England (1988).

[27] D. P. Shrout and D. A. Lightner, *Synthesis*, 1062 (1990).