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SYNTHESIS AND STEREOCHEMISTRY OF BILIRUBIN ANALOGS LACKING CARBOXYLIC ACIDS

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Abstract: Bilirubin analogs with propionic acid groups replaced by sec-butyl (1), 3-acetoxy-1-methylpropyl (2) or 3-acetamido-1-methylpropyl (3) were synthesized in enantiopure form and known absolute configuration. Although there are no propionic acid groups to act in stabilizing folded, ridge-tile conformations, the (S) stereogenic centers induce the pigments to adopt a left-handed (M) helicity — as evidenced by exciton circular dichroism spectra. © 1997 Elsevier Science Ltd.

INTRODUCTION

Bilirubin is a bis-dipyrrinone dicarboxylic acid formed in normal human metabolism of heme proteins^{1,2,3} at a rate of ~300 mg/day, mainly from the breakdown of red blood cells. High levels of serum bilirubin, which is lipophilic and intrinsically unexcretable, can cause irreversible neurologic damage. But the pigment is efficiently eliminated by the liver following uptake and enzymic conversion



to water-soluble glucuronides that are promptly secreted into bile. Impaired excretion of the glucuronides occurs in many types of hepatobiliary disease, but retention of native bilirubin is principally observed in newborn babies.¹⁻³ Accumulation of either native bilirubin or its glucuronides in the body is manifested in jaundice.

Bilirubin is conformationally flexible in solution, but one conformation is significantly more stable than all the others: a ridge-tile structure with intramolecular hydrogen bonds between the pyrrole and lactam functions of the dipyrrinone halves and the propionic carboxyl (or carboxylate) groups (Fig. 1).^{4,5} It can adopt porphyrinshaped conformations, but these are disfavored and of relatively high energy. It might also adopt the linear conformation shown above, but it is especially high energy.^{5,6} In fact, the ridge-tile shape is the only one that has been observed in crystals of bilirubin^{4a,b} and its carboxylate salts.^{4c} Spectroscopic studies, particularly NMR,^{7,8,9} supported by energy calculations,^{5a,6} strongly suggest that hydrogen-bonded ridge-tile conformers also prevail in solution. Individual ridge-tile conformers of bilirubin are conformational enantiomers that interconvert rapidly^{7,8} in solution through a succession of non-planar intermediates in which the hydrogen bonding network is never completely broken.^{5a,6} Yet, the energetically most favored ridge-tile conformation (interplanar angle, $\theta \sim 100^\circ$) is *not* rigid. It is flexible; and small, low energy rotations about the C(9)-C(10) and C(10)-C(11) bonds cause θ to close or open somewhat, while maintaining hydrogen bonding.^{5a,10} Large rotations, of course, close or open the conformation, book-like, breaking hydrogen bonds and leading to energetically disfavored conformations. Such large bond rotations are associated with the interconversion of mirror image ridge-tile conformations, a dynamic process occurring at an experimentallydetermined rate of ~5.4 sec⁻¹ at $37^{\circ}C^{7}$ over an experimentally-determined barrier of ~18-20 kcal/ mole.^{7c}

Bilirubin and its analog mesobilirubin-XIII α are thus a 50:50 mixture of equilibrating conformational enantiomers



FIGURE 1. Interconverting intramolecularly hydrogen-bonded enantiomeric conformers of bilirubin-IX α . The double headed arrows represent the dipyrrinone long wavelength electric transition moments (dipoles). The relative helicities (M, minus or P, plus) of the vectors are shown (inset) for each enantiomer. Hydrogen bonds are shown by dotted lines.

(Fig. 1), and displacement of the equilibrium toward one or the other of the enantiomers can be achieved by complexation with a chiral compound, such as quinine¹¹ or serum albumin,^{12,13} and observed by circular dichroism (CD) spectroscopy. Selective stabilization of one enantiomer can also be achieved through intramolecular nonbonded steric interactions, as has been observed when stereogenic centers are created by methyl substitution at either the α or the β carbons of the propionic acid chains.⁸ An especially useful allosteric model, ($\beta S, \beta' S$)-dimethylmesobilirubin-XIII α (22) has thus been used to probe intramolecular hydrogen bonding and conformational enantiomerism (Fig. 1). It is thought to adopt mainly the *M*-helical ridge-tile conformer in which non-bonded steric

interactions are minimized.^{8a} Such "intramolecular resolution," but absent the intramolecular hydrogen bonding, is also the subject of the current work. It occurred to us that the related optically active rubins (1-3) might also be used to probe pigment stereochemistry except in these models there is little (3) or no (1 and 2) opportunity for intramolecular hydrogen bonding. Yet, if nonbonded steric interactions remain important and if large CD Cotton effects are seen, it would seem likely that an important conformation-determining element in all bilirubins is simply the minimization of nonbonded steric interactions, which favors a ridge-tile shape.^{5,6}

RESULTS AND DISCUSSION



Synthesis. Bilirubin analogs 1-3 were prepared from optically active dipyrrinones 7-9, as outlined in the Synthetic Scheme. Thus, oxidative coupling using *p*-chloranil¹⁴ led to 56-88% yield of verdins 4-6, which were readily converted to rubins 1-3 in 67-89% yield using sodium borohydride. The optically active dipyrrinones (7-9) of known absolute configuration were prepared from a common precursor, monopyrrole 14, which had been synthesized and resolved earlier.^{8a} Conversion of 14 to 7 was achieved following (i) diborane reduction of the CO₂H group to CH₂OH in 13,¹⁵ (ii) conversion to tosylate 12,¹⁵ (iii) reaction of the tosylate with Zn and NaI in dimethoxyethane

SYNTHETIC SCHEME



^a NaBH₄, CH₃OH, THF; ^b p-chloranil, HCOOH; ^c NaOH, Δ , then HNO₃, then 5-bromomethylene-4-ethyl-3-methyl-2oxo-1*H*-pyrrole in CH₃OH, Δ ; ^d Ac₂O/pyr; ^e AcOH, DCCI, DMAP; ^f ϕ_3 P/THF-H₂O; ^gNaN₃/DMF; ^hNaI/acetone; ⁱZn, NaI, DME; ^jTsCl/pyr.; ^kBH₃•THF; ⁱCH₃CH₂OCOCl/Et₃N-THF, then NaBH₄ at -20°C; ^mNaOH/H₂O, then HCl.

to give 10, via iodide 11, and (iv) condensation of saponified 10 with 5-bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole.¹⁶ Dipyrrinone 8 was prepared in two steps in 88% yield from known dipyrrinone acid 16:¹⁵ (i) first by reduction of the mixed anhydride of 16 (from ethyl chloroformate) with NaBH₄ in 91% yield, (ii) then esterification of 17 using acetic anhydride in pyridine. Dipyrrinone 17 was converted to dipyrrinone 9 in 5 steps by the functional group modifications outlined: (i) 17 was converted to its tosylate (18) in 79% yield, using *p*-toluenesulfonyl chloride in pyridine; (ii) 18 was converted to its iodide (19) in 94% yield with NaI in acetone; (iii) iodide was displaced from 19 using NaN₃ in DMF to afford a 91% yield of azide 20; (iv) 20 was reduced to the amine with triphenylphosphine-THF-H₂O; and (v) the amine was acetylated *in situ* to form acetamide 9 in 93% yield for both steps using acetic acid, DCC and DMAP. The isolation and purification of amine 21 proved to be difficult; thus it was prepared and acetylated in one pot.

Circular Dichroism and Exciton Coupling. Moderately strong bisignate CD spectra (Fig. 2) were observed in CHCl₃ for 1-3, which may be viewed as bis-dipyrrinones. In clear contrast, the CD spectra of dipyrrinones 7-9 are extremely weak at best (Fig. 2, Table 1), in accord with expectations for a $\pi \rightarrow \pi^*$ CD transition perturbed by dissymmetric vicinal action.^{15,17} The few weak bisignate CD spectra found in 7-9 come from dimers^{15,17} formed in nonpolar solvents. The strong bisignate Cotton effects of 1-3 are characteristic of an exciton system in which the two dipyrrinone chromophores interact by coupling locally excited $\pi \rightarrow \pi^*$ transitions (electric transition dipole



FIGURE 2. Circular dichroism spectra of 1.5×10^{-5} M solutions of bilirubin analogs 1-3 and dipyrrinone 7 in chloroform at 22 °C. The number beside each curve corresponds to pigment 1, 2, 3 or 7.

coupling). The component dipyrrinone chromophores of the bichromophoric rubins have strongly-allowed longwavelength electronic transitions (Table 1) and interact through resonance splitting, *i.e.*, by electrostatic interaction of the local transition moment dipoles, which are oriented along the long axis of each dipyrrinone (Fig. 1). This intramolecular exciton splitting interaction produces two long wavelength transitions in the UV-vis spectrum and two corresponding bands in the CD spectrum. One band is higher in energy, and one is lower in energy, with the splitting being dependent on the strength and relative orientation of the dipyrrinone electric dipole transition moments. According to exciton chirality theory,¹⁸ a long wavelength negative, short wavelength positive CD couplet indicates a negative exciton chirality and a negative helical disposition of the relevant dipyrrinone long wavelength transition moments. Usually this corresponds to the *M*-helical enantiomeric type of Fig. 1,¹¹ but, as has been

shown previously, opening of the interplanar angle of either enantiomer without inverting molecular chirality can cause the transition moments to invert helicity and thus invert the Cotton effect signs of the couplet.^{5a}

		Circular Dichroism			UV-Visible	
Dipyrrinone	Solvent	$\Delta \epsilon^{max}(\lambda_1)$	λ_2 at $\Delta \epsilon = 0$	$\Delta \epsilon^{\max}(\lambda_3)$	€ ^{max}	λ(nm)
7 8 9	CCl ₄	<0.1 (400) +1.53 (397) +0.74 (400)	425 415	-0.80 (441) -0.65 (439)	43,400 44,100 42,000	(413) (410) (408)
7 8 9	Benzene	<0.1 (400) +0.85 (392) +1.47 (403)		-0.59 (441) -0.57 (440)	41,200 41,000 39,900	(411) (409) (408)
7 8 9	CHCl ₃	<0.1 (400) +0.82 (399) -1.63 (407)	 416	<0.1 (440) +2.89 (439)	32,900 34,000 33,200	(410) (407) (406)
7 8 9	(CH ₃) ₂ CO	<0.1 (400) +0.66 (394) <0.1 (400)	 	<0.1 (440) <0.1 (440)	35,200 35,300 35,000	(405) (403) (403)
7 8 9	СН ₃ ОН	<0.1 (400) <+0.1 (390) <+0.1 (400)			37,800 38,800 38,600	(416) (413) (414)
7 8 9	CH ₃ CN	<0.1 (400) <+0.1 (390) <+0.1 (400)			34,100 34,200 34,000	(403) (401) (400)

TABLE 1. Circular Dichroism and UV-Visible Spectral Data for Dipyrrinones 7-9.

Circular Dichroism and Conformation. The effectiveness of the $8^{1}S_{12}S_{12}$ methyls of 1-3 in displacing the M ≠ P conformational equilibrium may be seen in their CD spectra (Fig. 2). Unlike simple dipyrrinones, the CD spectra of 1-3 are indicative of strong exciton coupling, 5,8,11,18 and the signed order of the Cotton effects implies a negative (M-helical) orientation of the component dipyrrinone long wavelength electric transition dipole moments. This means the rubins must adopt preferentially a chiral conformation without the benefit of conformational stabilization provided by intramolecular hydrogen bonds (Fig. 1).^{5,11} When carboxylic acid groups are present and properly located to stabilize the ridge-tile conformations, more intense exciton CD Cotton effects are seen ---as in the optically active, intramolecularly hydrogen-bonded ($\beta S, \beta' S$)-dimethylmesobilirubin-XIII α (22) (Fig. 3).^{5a,8a} It is clear from Fig. 3 that (i) diacid 22 and 1-3 share the same signed-sequence for their bisignate CD Cotton effects, and (ii) while the 1 and 2 have Cotton effects with similar magnitudes, they are much less intense than either 22 or 3, which exhibit Cotton effects of comparable intensity. The intensity differences appear to be linked to the fact that the diacid 22 can stabilize the *M*-helicity ridge-tile enantiomer using a full complement of six intramolecular hydrogen bonds (Fig. 1); whereas, 1 and 2 cannot, and 3 can participate in minimal intramolecular hydrogen bonding using the two acetamide NHs. Although amide carbonyls are thought to be better hydrogen bond acceptors than ester carbonyls, the amide carbonyl of 3 and ester 2 are probably too far removed to be involved effectively in hydrogen bonding.



FIGURE 3. Circular dichroism spectra of 1.5×10^{-5} M solutions of bilirubin analogs (1-3) and ($\beta S, \beta' S$)-dimethylmesobilirubin-XIII α (22) in acetonitrile at 22°C. The number beside each curve corresponds to pigment 1, 2, 3 or 22.

The sensitivity of the CD data to changes in solvent may be noted in Table 2: 1 and 2 are only moderately sensitive to vastly different solvent polarity (dipoles) and hydrogen-bonding ability. In contrast, although the $\Delta \epsilon$ values for 3 tend to be larger than those of 1 and 2, they range from a high of $\Delta \epsilon_{436}^{max} - 322$ in cyclohexane to $\Delta \epsilon_{419}^{max} - 74$ in CHCl₃, to a low of $\Delta \epsilon_{426}^{max} - 22$ in (CH₃)₂SO. The lower $\Delta \epsilon$ values of 3 tend to appear in solvents that participate in hydrogen bonding, and the highest values are not restricted to nonpolar solvents. On the other hand, with a full complement of six intramolecular hydrogen bonds (Fig. 1), as in diacid 22, the $\Delta \epsilon$ values resist solvent perturbation over the entire scope of solvents and remain within 10-20% of the value seen in benzene ($\Delta \epsilon_{434}^{max} - 362$) — except in the special case of (CH₃)₂SO, where $\Delta \epsilon_{422}^{max} - 24$.^{8a} Nevertheless, the data of Table 2 suggest that even the most minimal intramolecular hydrogen bonding (as in 3) can stabilize a particular ridge-tile shape in the absence of strong solvent perturbation and that hydrogen bonding is not essential to the diastereoselectivity of the *M*-helical conformer over the *P*. Diastereoselectivity apparently derives exclusively from the pigment's simply adopting a shape that minimizes intramolecular nonbonded steric interactions. For the (8¹S,12¹S) configuration of 1-3 and 22 this means a preference for the *M*-helical conformer of Fig. 1.^{5,8a,11}

Consistent with these conclusions, exciton analysis⁵ of the UV-visible spectra (Table 2) suggests that the folded enantiomeric conformers probably favor a ridge-tile helical shape rather than a more extended shape. Unlike bilirubin and its analog 22, which have UV-vis spectra with a more dominant long wavelength exciton component, the spectra of 1-3 indicate that the shorter wavelength exciton component is more intense than the long wavelength component in most of the solvents studied, especially for 1 and 2, but less so for 3. These data suggest the prevalence of conformations for 1-3 with an interplanar angle smaller than the 100° found in 22 and bilirubin, e.g., a more acute ridge-tile tending toward a helical conformation, or to a distorted ridge-tile.¹⁹ But it is difficult to predict with certainty the size of the interplanar angle from the UV-vis or CD data.

Stereochemistry from NMR: Nuclear Overhauser Effects. Additional experimental evidence for a folded conformation of *M*-helicity in 1-3 comes from NMR studies in CDCl₃. A syn-Z configuration of the dipyrrinones is established by: (i) strong ${}^{1}H{}^{1}H{}$ -NOEs between the pyrrole and lactam NHs and (ii) NOEs between the vinyl hydrogens at C(5)/C(15) and the methyls at C(7)/C(13) and the C(3)/C(17) ethyl CH₂ groups. NOEs between the ${}^{8}/12{}^{1}$ CHs and the C(10) CH₂, and between the CH₃s at ${}^{8}/12{}^{1}$ and the C(7)/C(13) CH₃s indicates a preference

Circular Dichroism			UV-Visible			
	$λ_2$ at Δε=0	$\Delta \epsilon^{\max}(\lambda_3)$	€ ^{max}	λ(nm)	€ ^{max}	λ(nm)
	393	-46.3 (440)	70,300	376	12,600	434 ^{sh}
	391	-34.0 (423)	76,300	375	15,200	426 ^{sh}
)	410	-322.0 (436)	60,700	400	35,800	433
	394	-35.0 (439)	59,700	378		
	402	-18.2 (433)	63,800	377		
	391	-116.8 (418)	51,100	391	49,700	411
	394	-45.1 (440)	67,700	380	14,000	432 ^{sh}
	391	-41.3 (421)	72,700	377	15,300	430 ^{sh}
	392	-154.1 (419)	49,500	389	38,400	415 ^{sh}
	394	-40.1 (433)	64,700	379	15,300	430 ^{sh}
	389	-58.3 (416)	62,300	378		
	392	-73.7 (419)	48,600	391	46,100	408 ^{sh}

69,300

76,400

51,900

64,000

69,700

53,100

47,300

51,900

50,600

46,700

52,200

51,000

66,900

73,700

52,400

46,400

53,400

50,600

43,800

52,000

47,300

49,500

54,400

48,700

374

374

387

376

374

389

394

393

396

395

393

394

375

373

388

392

389

391

398^{sh}

398^{sh}

397^{sh}

393

390

394

15,300

46,600

20,600

48,600

46,000

52,500

50,600

47,300

51,800

50,100

13,300

45,900

50,300

53,700

49,700

55,400

58,600

54,800

42,700

46,900

47,100

TABLE 2. Comparison of CD and UV Spec

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-40.3(432)

-21.2(418)

-170.5 (417)

-40.5(425)

-21.9 (419)

-198.2(419)

-21.1(434)

-32.0 (425)

-52.8 (426)

-20.0(430)

-32.8(424)

-65.4 (425)

-37.7 (429)

-23.9(421)

-190.9 (417)

-24.2 (425)

-26.5 (421)

-61.6 (422)

-29.7 (428)

-28.7(423)

-22.2 (426)

-20.8(432)

-24.2 (427)

-44.5 (425)

Pig-

ment

1

2

3

1

2

3

1

2

3

1

2

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1

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3

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2

3

Solvent

Cyclohexane

1,4-Dioxane

Benzene

CHCl₃

CH₃CO₂C₂H₅

(CH₃)₂CO

CH₃CH₂OH

CH₃OH

CH₃CN

(CH₃)₂NCHO

(CH₃)₂SO

CH₃NHCHO

εa

2.0

2.2

2.3

4.7

6.0

20.7

24.3

32.6

36.2

36.7

46.5

181.2

 $\Delta \epsilon^{\max}(\lambda_1)$

+50.4 (372)

+37.3 (369)

+157.5 (397)

+35.9 (373)

+15.2 (369)

+69.5 (377)

+42.8(374)

+39.1(371)

+91.4 (378)

+42.2 (373)

+39.3 (373)

+50.4 (376)

+46.2(370)

+24.7 (369)

+103.1 (375)

+44.8(370)

+23.2(373)

+130.5 (376)

+17.7 (388)

+26.0 (379)

+45.7 (382)

+17.3 (382)

+26.2 (379)

+53.7 (380)

+41.7 (370)

+27.0 (370)

+133.8(375)

+17.7 (376)

+24.4(378)

+45.6 (379)

+19.7 (386)

+26.5 (382)

+31.0 (384)

+16.6 (387)

+20.2(383)

+37.4 (379)

for the *M*-helicity conformation in the $8^{1}S_{1}2^{1}S$ enantiomer. Here the 8^{1} , 12^{1} -methines are brought into close proximity to the C(10) CH₂ hydrogens, and the 8^{1} , 12^{1} methyls lie near the methyls at C(7) and C(13). (In the *P*helicity conformation the opposite occurs, with the 8^{1} , 12^{1} methyls lying close to the C(10) CH₂, and the 8^{1} , 12^{1} methines lying close to the C(7)/C(13)-methyls.) These data are quite similar to those seen in 22: the signals for

424^{sh}

410^{sh} 422^{sh}

411^{sh}

425

420

419

425

417

417

430^{sh}

412^{sh}

423

417

417

430

425

428

425

418

420

the C(7)/C(13) methyls and the β , β' -methyls are also related by a strong NOE, as are the β -CHs and the C(10) CH₂. In **22** other NOEs are found between the lactam and pyrrole NHs, between C(5)/C(15) hydrogens and the C(3)/C(17)-ethyl and C(7), C(13)-methyl groups, confirming that the dipyrrinones adopt a *syn-Z* conformation. Direct evidence for an intramolecularly hydrogen-bonded conformation of **22** comes from irradiation of the carboxylic acid hydrogen (COOH) and observing a 4.5% NOE on the lactam NHs. These findings are consistent with the predictions of the exciton chirality rule and the data of Table 2.

Interestingly, weak NOEs were also detected between the methyls at C(2)/C(18) and (i) the C(10) CH₂ and (ii) the pyrrole NHs of 1-3. Such NOEs may be taken to indicate the presence of dimers formed by *inter*molecular hydrogen bonding between dipyrrinone units. Similar NOEs were reported previously for dipyrrinones such as kryptopyrromethenone and methyl xanthobilirubinate, where dipyrrinone to dipyrrinone dimeric association through intermolecular hydrogen bonding prevailed in nonpolar solvents such as chloroform, but not in more polar solvents, such as dimethylsulfoxide.²⁰ This is consistent with the dimeric structure suggested for bilirubin dimethyl ester based on conclusions from NOE and vapor pressure osmometry studies.¹⁹ However, unlike dipyrrinones, little if any effect of dilution is seen on the NH chemical shifts (Table 3). Thus, one may assume a dimer for 1 with $K_{assoc} \gg 10^4$, inasmuch as K_{assoc} for dipyrrinones is ~25,000 M in CDCl₃ at 22°C.^{19,20} In CDCl₃ - 10% (CD₃)₂SO solvent, the intermolecular NOEs vanish, while the same intramolecular NOEs as above are maintained. The small amount of added (CD₃)₂SO is sufficient to disrupt the dimeric association but is known to be insufficient to disengage intramolecular hydrogen bonding in bilirubin.²¹ It is important to note that despite the potential for dimer formation in nonpolar solvents, the CD spectra of 1-3 maintain the same Cotton effect signs over a wide range of solvent polarity, with the magnitudes of 1 and 2 being nearly the same over the range (Table 2). The origin of the exciton appears thus to be largely *intra* rather than *inter*molecular.

Concentration [M]			$0 \xrightarrow{1}{N} \xrightarrow{5}{N} \xrightarrow{9}{10} \xrightarrow{12}{11} \xrightarrow{15}{N} \xrightarrow{19}{0} \xrightarrow{19}{10}$		
	Lactam NH	Pyrrole NH	Lactam NH	Pyrrole NH	
2 × 10 ⁻²	11.30	10.28	10.77	10.17	
5 × 10 ⁻³	11.16	10.18	10.77	10.17	
5 × 10 ⁻⁴	10.67	9.88	10.77	10.17	
5 ×10 ⁻⁵	10.33	9.73	10.77	10.17	

TABLE 3. Concentration Dependence of Pyrrole and Lactam NH Chemical Shifts in CDCl₃ Solutions.

Conformational Analysis from Molecular Mechanics Calculations. A conformational energy map correlating a multiplicity of bilirubin conformations may be created from the results of molecular dynamics calculations.⁵ In this study, we determined the range of conformations created by rotating a rubin's two dipyrrinones independently about the C(9)-C(10) and C(10)-C(11) bonds in steps of 10°. The conformations are defined by the

corresponing torsion angles, $\phi_1 = N(22)-C(9)-C(10)-C(11)$ and $\phi_2 = C(9)-C(10)-C(11-N(23))$, where $\phi_1 = \phi_2 = 0^\circ$ corresponds to a planar porphyrin-like shape, which is very high energy, and $\phi_1 = \phi_2 \sim 180^\circ$ corresponds to the linear shape (Synthetic Scheme), which is also very high energy.⁵ For 1, the conformational energy map (Fig. 4) shows that these very high energy conformations lie atop full peaks surrounded by valleys or canyons containing several potential energy wells that correspond to global and local minima. The global minimum conformation found at $\phi_1 = \phi_2 \sim -50^\circ$ has the typical ridge-tile shape (*M*, Figs. 5 and 1), albeit with twisted rather than planar dipyrrinones. Two local minima, essentially isoenergetic with the global minimum, lie nearby at $\phi_1 \sim -110^\circ$, $\phi_2 \sim +50^\circ$) and ($\phi_1 \sim -60^\circ$, $\phi_2 \sim +140^\circ$) — along a canyon or path connecting the *M*-helicity ridge-tile with the *P*-helicity ridge-tile shape local minimum at $\phi_1 = \phi_2 \sim -130^\circ$) and ($\phi_1 = \phi_2 \sim +120^\circ$) lie nearby the *M* and *P*-helicity ridge-tile minima, respectively, and correspond to nearby enantiomeric, extended or stretched conformations (Fig. 5). Subject to modification due to solvation effects, the data suggest that 1 adopts mainly the ($\phi_1 = \phi_2 \sim -50^\circ$), ($\phi_1 \sim -110^\circ$, $\phi_2 \sim +50^\circ$) and ($\phi_1 \sim -60^\circ$, $\phi_2 \sim +50^\circ$) and ($\phi_1 \sim -60^\circ$, $\phi_2 \sim +140^\circ$) and ($\phi_1 \sim -60^\circ$, $\phi_2 \sim +140^\circ$).



FIGURE 4. Conformational energy surface (left) and its energy scale (below), and contour map (right) and its energy scale for mesobilirubin analog 1 conformers generated by rotating its two dipyrrinones independently about the C₉-C₁₀ and C₁₀-C₁₁ carbon-carbon single bonds (ϕ_1 and ϕ_2 , respectively) in steps of 10°. The global minimum (set to 0.0 kcal/mole) is found near $\phi_1 = \phi_2 \sim -50^\circ$, corresponding to the *M*-helicity enantiomer. Local minima are found near $\phi_1 \sim -110^\circ$, $\phi_2 \sim$ +50°; $\phi_1 \sim -60^\circ$, $\phi_2 \sim +140^\circ$, $\phi_1 = \phi_2 \sim +70^\circ$ (*P*-helicity ridge-tile) $\phi_1 = \phi_2 \sim +120^\circ$, and $\phi_1 = \phi_2 \sim -130^\circ$, lying 0.1, 0.3, 1.1, 1.1 and 2.2 kcal/mole above the global minmum. Data are from molecular dynamics simulations using SYBYL® (Tripos Assoc.) on an Evans & Sutherland ESV-10 workstation according to ref. 5. The energy displays were created using WingzTM (Informix).

Significantly, the global minimum conformation of 1 has nearly the same shape as the global minimum conformation of bilirubin (Fig. 1), or more specifically that of analog 22: the *M*-helicity ridge-tile. The major geometric difference lies in the slightly twisted dipyrrinones of 1; whereas, in the intramolecularly hydrogen-bonded analog (22) the dipyrrinones are nearly planar. In both 1 and 22 the global minimum conformation is one where nonbonded steric repulsions are minimized, but in 22 this conformation is further stabilized by six intramolecular hydrogen bonds, which is significant (~20 kcal/mole). Thus, in the conformational energy map of 22,^{8a} the energy wells corresponding to the *M*-helicity global and *P*-helicity local minima are much deeper, and the interconversion barriers are higher (~20 kcal/mole) between the *M*-helicity global minimum and the *P*-helicity local minimum (which lies ~4.3 kcal/mole above the global minimum).⁵ Thus, 22 exhibits very intense CD (Fig. 3). In 1, on the other hand, interconversions barriers between global and local minima are much lower, and the energy differences among the minima are also smaller. This means that the CD spectrum (Fig. 3) seen for 1 is actually the population weighted sum of the CDs of each contributing conformation — according to molecular dynamics computations, mainly those corresponding to ($\phi_1 = \phi_2 \sim -50^\circ$), ($\phi_1 \sim -110^\circ$, $\phi_2 \sim +50^\circ$) and ($\phi_1 \sim -60^\circ$, $\phi_2 \sim +140^\circ$). Since 1 exhibits a CD of ~15% of the intensity of 22, it would appear that while the contribution of the *M*-chirality conformation at ($\phi_1 = \phi_2 \sim -50^\circ$) dominates the CD, the net contributions of the other contributing conformations are nearly self-cancelling, as would be expected from the structures and relative energies of Fig. 5.



FIGURE 5. Ball and stick conformational representations relating the ridge-tile shaped *M*-helicity global minimum to local minima. Conformational representatives were created using Müller and Falk's "Ball and Stick." Structures are identified by torsion angles (ϕ_1 , ϕ_2) and their relative energies in brackets, [kcal/mole]. The energy barriers in kcal/mole are given next to the arrows.

Although the foregoing molecular dynamics conformational analysis is for *unsolvated* 1 and 22, it may be used to gain insight into the probable shape of the pigment when it is monomeric in solution, as in the higher

dielectric solvents of Table 2. In nonpolar solvents a dimer is apparently dominant; so we modelled dimers of 1 in order to extract information on the stereochemistry of each pigment unit. These results are summarized in the dimeric structures of Figure 6. In the energy-minimum dimer, the two monomer units have essentially identical conformations, with $\phi_1 \sim -95^\circ$, $\phi_2 \sim +13^\circ$ and $\phi_1 \sim -95^\circ$, $\phi_2 \sim +100^\circ$ corresponding to an *M*-helical rubin. These conformations are predicted^{5a} to exhibit strong negative exciton coupling CD spectra. For comparison, the dimer formed of *P*-helicity monomer units of 1 (Figure 6) lies 0.7 kcal/mole higher energy, and the two monomer units have essentially identical conformations, with $\phi_1 \sim +100^\circ$, $\phi_2 \sim -21^\circ$ and $\phi_1 \sim +100^\circ$, $\phi_2 \sim -27^\circ$. These conformations are predicted to exhibit strong positive exciton coupling Cotton effects. And the dimer formed from *M* and *P*-helical components cannot adopt more than two hydrogen bonds and is higher energy. Since the *M*-helical dimer is slightly favored over the *P*, the observed moderate, negative exciton Cotton effects observed in nonpolar solvents (Table 2) are supported by this molecular mechanics analysis of conformation.



FIGURE 6. Ball and stick representations for low-energy dimers of 1. (Left) Minimum energy dimer of 1 held by intermolecular hydrogen bonds between the component dipyrrinones. In each component of the dimer, $\phi_1 \sim -95^\circ$, $\phi_2 \sim +13^\circ$, corresponding to an *M*-helicity. (Right) Higher energy dimer of 1 where each component adopts a *P*-helicity with $\phi_1 \sim +100^\circ$, $\phi_2 \sim -27^\circ$. Hydrogens are removed from carbons for clarity of representation. Hydrogen bonds are shown by hatchmarks. In each dimer, bold numbering corresponds to one monomer unit; italics numbering corresponds to the second monomer unit.

CONCLUDING COMMENTS

Intramolecular hydrogen bonding, which is characteristic of natural bilirubin, is known to be a dominant force in determining the pigment's conformation (Fig. 1). The current study shows that when the propionic acids at C(8) and C(12) are replaced by alkyl or ether groups having stereogenic centers at 8^1 and 12^1 , 1 and 2 are forced by nonbonded steric interactions to adopt an *M*-helicity conformation. When residual intramolecular hydrogen bonding is possible, as in amide 3, the *M*-helicity shaped ridge-tile conformer is dominant. Molecular dynamics computations support the interesting and surprising result derived from spectroscopy: the *M*-helicity conformation is clearly favored, even in the absence of intramolecular hydrogen bonding.

EXPERIMENTAL

General. All circular dichroism spectra were recorded on a JASCO J-600 instrument, and all UV-vis spectra were recorded on a Perkin Elmer Lambda 12 or Cary 219 spectrophotometer. NMR spectra were obtained on a GN-300 or Varian Unity Plus spectrometers operating at 300 MHz and 500 MHz, respectively. CDCl₃ solvent was used throughout and chemical shifts were reported in δ ppm referenced to residual CHCl₃ ¹H signal at 7.26 ppm and ¹³C-signal at 77.00 ppm. J-modulated spin-echo experiments (*A*ttached *P*roton *T*est) were used to obtain the ¹³C-NMR assignments. Optical rotations were measured on a Perkin Elmer model 141 polarimeter. HPLC analyses were carried out on a Perkin Elmer Series 410 high-pressure liquid chromatograph with a Perkin Elmer LC-95 UV-vis spectrophotometric detector (set at 420 nm) equipped with a Beckman Altex ultrasphere IP 5 µm C-18 ODS column (25 × 0.46 cm) kept at 34°C. The flow rate was 1.0 mL min⁻¹ and the mobile phase was 0.1 M di-*n*-octylamine acetate buffer in 5% aqueous methanol (pH 7.7 at 25°C). Radial chromatograph was carried out on Merck Silica gel PF₂₅₄ with CaSO₄ preparative layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA) with 1, 2 or 4 mm thick rotors. Melting points were determined on a Mel-Temp capillary apparatus and are uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ.

The spectral data were obtained in spectral grade solvents (Aldrich or Fisher). HPLC grade solvents were dried and purified following standard procedures.²² All commercial reagents were from Aldrich or ACROS.

The starting compounds: optically pure (+)-3(S)-(2,4-dimethyl-5-ethoxycarbonyl-1*H*-pyrrol-3-yl) butanoic acid (14);^{8a} (+) methyl 3(S)-(4-ethyl-3,8,10-trimethyl-2-oxo-1,11-dihydrodipyrrin-9-yl)butanoate (15);^{8a} (+)-3(S)-(2,4-dimethyl-5-ethoxycarbonyl-1H-pyrrol-3-yl)butan-1-ol (13) and its *p*-toluenesulfonate (12);¹⁵ and (-)-3(S)-(4-ethyl-3,8,10-trimethyl-2-oxo-1,11-dihydrodipyrrin-9-yl)butanoic acid (16)¹⁵ were synthesized as described previously.

(+) Ethyl 3,5-dimethyl-4-[1(*S*)-methylpropyl]-*1H*-pyrrole-2-carboxylate (10). A mixture of 3.93 g (10 mmol) of tosylate 12, 7.50 g (50 mmol) of NaI, 6.54 g (100 mg-A) Zn, 100 mL of 1,2-dimethoxyethane, and 4 mL of H₂O were combined and heated under reflux for 3h. After cooling, the mixture was filtered and the filtered solid was washed with 3 × 30 mL of CH₂Cl₂. The combined filtrates were washed with 5% HCl (2 × 35 mL), H₂O (3 × 50 mL) then dried over anhydrous MgSO₄ and filtered. The solvent was removed under vacuum to afford the crude product (2.22 g, quantitative yield), which was used in the next step without further purification. An analytical sample was obtained after recrystallization from ethyl acetate-hexane. It had mp 95-96°C; $[\alpha]_D^{20} + 24.0°$ (*c* 1.3, C₂H₅OH); ¹H-NMR: δ 0.81 (3H, t, J=7.4 Hz), 1.22 (3H, d, J=7.2 Hz), 1.34 (3H, t, J=7.1 Hz), 1.55-1.65 (2H, m), 2.22 (3H, s), 2.31 (3H, s), 2.57-2.64 (1H, m), 4.29 (2H, q, J=7.1 Hz), 8.58 (1H, br.s) ppm and ¹³C-NMR: δ 11.06, 12.68, 12.71, 14.58, 20.41, 29.42, 32.45, 59.49, 116.56, 125.81, 126.98, 129.00, 161.76 ppm. Anal. Calcd. for C₁₃H₂₁NO₂ (223.3): C, 69.92; H, 9.48; N, 6.27.

Found: C, 69.89; H, 9.53; N, 6.35.

(+)-3(S)-(4-Ethyl-3,8,10-trimethyl-2-oxo-1,11-dihydrodipyrrin-9-yl)butan-1-ol (17). To an N₂-blanketed, cooled (-20°C) solution of 1.58 g (5 mmol) of acid 16 and 0.84 mL (6 mmol) of dry triethylamine in 40 mL of dry THF was added 0.60 mL (6 mmol) of ethyl chloroformate. The mixture was stirred for 15 min and the resulting precipitate was filtered and washed with 2×5 mL portions of dry THF under N₂ protection. To the filtrate was added sodium borohydride (230 mg, 6 mmol) at -20°C, followed by 4 mL of dry methanol. The mixture was

stirred for 2h at -20° C, and the reaction was quenched with 10 mL of H₂O. The mixture was diluted with 250 mL of CHCl₃, washed with H₂O (2 × 200 mL), dried (anhydrous Na₂SO₄) and filtered. The solvent was removed under vacuum, and the resulting product was triturated with 5 mL of CH₃OH and 20 mL of dry ether to afford 1.38 g, 91% of pure alcohol **17**, which was used in the next step. An analytically pure sample was obtained after radial chromatography (4% CH₃OH/CH₂Cl₂ eluent) and recrystallization from CH₃OH/Et₂O. It had mp 226-227 °C (decomp.), $[\alpha]_D^{20}$ +54.3° (*c* 0.5, CHCl₃); ¹H-NMR: δ 1.17 (3H, t, J=7.6 Hz), 1.24 (1H, br.t), 1.28 (3H, d, J=7.2 Hz), 1.85-1.91 (2H, m), 1.94 (3H, s), 2.18 (3H, s), 2.46 (3H, s), 2.55 (2H, q, J=7.6 Hz), 2.87-3.00 (1H, m) 3.55-3.63 (2H, m), 6.14 (1H, s), 10.32 (1H, br.s), 11.30 (1H, br.s) ppm; and ¹³C-NMR: δ 8.55, 10.39, 12.63, 15.05, 17.96, 21.04, 27.58, 39.18, 62.11, 100.93, 122.35, 122.45, 123.91, 124.22, 127.15, 131.03, 148.37, 174.11 ppm. Anal. Calcd. for C₁₈H₂₆N₂O₂ (302.4): C, 71.49; H, 8.67; N, 9.26. Found: C, 70.95; H, 8.63; N, 8.92.

(-)-3(S)-(4-Ethyl-3,8,10-trimethyl-2-oxo-1,11-dihydrodipyrrin-9-yl)butan-1-ol *p*-toluenesulfonate (18). A mixture of 1.31 g (4.3 mmol) of alcohol 17, 22 mL of dry CH_2Cl_2 , and 1.2 mL (8.6 mmol) of Et_3N was cooled with ice bath and it was added 1.65 g (8.6 mmol) of *p*-toluenesulfonyl chloride over 20 min. After stirring 14h at room temperature, the reaction mixture was diluted with 300 mL of $CHCl_3$ and washed with 1% of HCl (100 mL) then water (3 × 200 mL). After drying over anhydrous Na_2SO_4 and filtering, the solvent was evaporated under vacuum. The crude product was purified by radial chromatography (eluent: 1.5-3% CH_3OH in CH_2Cl_2) and recrystallized from $CHCl_3$ - CH_3OH to afford 1.55 g (79%) of tosylate 18. It had mp 160-162°C; $[\alpha]_D^{20} - 40.3^{\circ}$ (*c* 0.4, $CHCl_3$); ¹H-NMR: δ 1.18 (3H, t, J=7.6 Hz), 1.22 (3H, d, J=7.0 Hz), 1.95 (3H, s), 1.92-1.99 (2H, m), 2.08 (3H, s), 2.36 (3H, s), 2.39 (3H, s), 2.55 (2H, q, J=7.6 Hz), 2.88 (1H, dq, J=7.0; 7.0 Hz), 3.82-3.90 (1H, m), 3.96-4.03 (1H, m), 6.10 (1H, s), 7.29 (2H, d, J=8.2 Hz), 7.72 (2H, d, J=8.2 Hz), 10.28 (1H, br.s), 11.35 (1H, br.s) ppm; and ¹³C-NMR: δ 8.56, 10.30, 12.48, 15.06, 17.96, 20.68, 21.55, 26.86, 35.28, 69.59, 100.90, 122.38, 122.43, 122.56, 124.16, 127.12, 127.79, 129.71, 131.14, 133.14, 144.54, 148.43, 174.13 ppm.

Anal. Calcd. for C₂₅H₃₂N₂O₄S (456.6): C, 65.76; H, 7.06; N, 6.14. Found: C, 65.49; H, 7.09; N, 6.06.

(+)-3(*S*)-(4-Ethyl-3,8,10-trimethyl-2-oxo-1,11-dihydrodipyrrin-9-yl)-1-iodobutane (19). A mixture of 1.37 g (3 mmol) of tosylate 18, 4.50 g (30 mmol) of NaI, and 90 mL of dry acetone was stirred and heated at reflux vigorously for 3h. The solvent was evaporated under vacuum, and the residue was partitioned between 300 mL of CHCl₃ and 200 mL of H₂O. The organic layer washed with H₂O (3 × 100 mL), dried over anhydrous Na₂SO₄ and filtered. The solvent was evaporated under vacuum, and the resulting crude product was recrystallized from CHCl₃-CH₃OH to afford 1.16 g (94%) of iodide 19. It had mp 178-180°C; $[\alpha]_D^{20}$ +87.6°(c 0.5, CHCl₃); ¹H-NMR: δ 1.17 (3H, t, J=7.6 Hz), 1.27 (3H, d, J=7.1 Hz), 1.95 (3H, s), 2.01-2.11 (1H, m), 2.13-2.24 (1H, m), 2.18 (3H, s), 2.48 (3H, s), 2.55 (2H, q, J=7.6 Hz), 2.85-2.95 (1H, m), 3.02 (1H, dt, J=9.1; 7.7 Hz), 3.17 (1H, dt, J=9.1; 7.0 Hz), 6.14 (1H, s), 10.32 (1H, br.s), 11.30 (1H, br.s); and ¹³C-NMR: δ 6.18, 8.57, 10.51, 12.79, 15.05, 17.96, 20.40, 31.43, 40.14, 100.88, 122.38, 122.49, 122.65, 124.25, 127.18, 131.29, 148.39, 174.14 ppm. Anal. Calcd. for C₁₈H₂₅IN₂O (412.3): C, 52.43; H, 6.11; N, 6.79.

Found: C, 52.37; H, 6.05; N, 6.63.

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(+)-3(S)-(4-Ethyl-3,8,10-trimethyl-2-oxo-1,11-dihydrodipyrrin-9-yl)-1-azidobutane (20). A mixture of 1.03 g (2.5 mmol) of iodide 19, 813 mg (12.5 mmol) of sodium azide, and 55 mL of dry dimethylformamide was stirred at 50°C for 4h. After cooling, it was diluted with 200 mL of CHCl₃, washed with H₂O (4 × 200 mL) and dried over anhydrous Na₂SO₄. After filtration, the solvent was evaporated under vacuum. The resulting crude product was recrystallized from CHCl₃-CH₃OH to afford 745 mg (91%) of azide 20. It had mp 179-181°C; $[\alpha]_D^{20}$ +91.8° (c 0.5, CHCl₃); ¹H-NMR: δ 1.18 (3H, t, J=7.6 Hz), 1.29 (3H, d, J=7.2 Hz), 1.82-1.90 (2H, m), 1.95 (3H, s), 2.16 (3H, s), 2.45 (3H, s), 2.55 (2H, q, J=7.6 Hz), 2.83-2.96 (1H, m), 3.12 (1H, dt, J=12.1; 7.5 Hz), 3.26 (1H, dt, J=12.1; 6.7 Hz), 6.14 (1H, s), 10.34 (1H, br.s), 11.33 (1H, br.s) ppm; and ¹³C-NMR: δ 8.54, 10.33, 12.54, 15.05, 17.92, 20.76, 27.88, 35.15, 50.13, 100.86, 122.32, 122.40, 122.89, 124.16, 127.09, 131.08, 148.35, 174.10 ppm. Anal. Calcd. for C₁₈H₂₅N₅O (327.4): C, 66.03; H, 7.70; N, 21.39. Found: C, 65.96; H, 7.69; N, 21.40.

3-(4-Ethyl-3,8,10-trimethyl-2-oxo-1,11-dihydrodipyrrin-9-yl)-1-butyl amine (21). A mixture of 164 mg (0.5 mmol) of racemic azide **20**, 197 mg (0.75 mmol) triphenyl phosphine, 4 mL of THF and 0.3 mL of H₂O was heated under reflux for 3h. The solvents were evaporated under vacuum. Recrystallization of the residual from benzene-diethyl ether afforded 52 mg (34%) of amine **21**. It had mp 212-214°C; ¹H-NMR: δ 1.15 (2H, very br.s), 1.17 (3H, t, J=7.6 Hz), 1.26 (3H, d, J=7.1 Hz), 1.72-1.80 (2H, m), 1.94 (3H, s), 2.16 (3H, s), 2.45 (3H, s), 2.55 (2H, q, J=7.6 Hz), 2.64 (2H, br.t, J=7.4 Hz), 2.80-2.87 (1H, m), 6.14 (1H, s), 10.33 (1H, br.s), 11.34 (1H, br.s) ppm; and ¹³C-NMR: δ 8.55, 10.39, 12.69, 15.08, 17.94, 21.00, 28.37, 40.57, 41.06, 100.99, 122.17, 122.23, 124.30, 124.39, 126.93, 130.93, 148.29, 174.04 ppm. This amine was generated *in situ* and reacted to form **9**.

(+)-4-Ethyl-9-[1(*S*)-methylpropyl]-3,8,10-trimethyl-2-oxo-1,11-dihydrodipyrrinone (7). A mixture of 1.56 g (7 mmol) of ethyl ester 10, 1.40 g (35 mmol) of NaOH, 15 mL of ethanol, and 5 mL of water was heated at reflux for 3h. The solvents were removed under vacuum. To the residue was added 35 mL of methanol, and the mixture was carefully acidified with conc. HNO₃ at 0°C. 5-Bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole¹⁶ (1.51 g, 7 mmol) was added, and the mixture was heated at reflux for 7h. It was chilled overnight at -20° C. The resulting precipitate was filtered and washed with cold methanol. The crude product was dissolved in CH₂Cl₂, filtered, evaporated and recrystallized from chloroform-methanol to afford 1.42 g (71%) of bright yellow dipyrrinone 7. It had mp 241-242°C; $[\alpha]_D^{20}$ +53.3° (*c* 0.6, CHCl₃); ¹H-NMR: δ 0.84 (3H, t, J=7.3 Hz), 1.17 (3H, t, J=7.6 Hz), 1.25 (3H, d, J=7.1 Hz), 1.57-1.66 (2H, m), 1.95 (3H, s), 2.16 (3H, s), 2.45 (3H, s), 2.55 (2H, q, J=7.6 Hz), 2.59-2.67 (1H, m), 6.15 (1H, s), 10.28 (1H, br.s), 11.30 (1H, br.s) ppm; and ¹³C-NMR: δ 8.53, 10.34, 12.73, 12.83, 15.05, 17.95, 20.55, 29.58, 32.69, 101.15, 122.03, 122.15, 124.66, 124.92, 126.79, 131.18, 148.25, 174.03 ppm.

Anal. Calcd. for C₁₈H₂₆N₂O (286.4): C, 75.48; H, 9.15; N, 9.78. Found: C, 74.97; H, 9.16; N, 9.75.

(+)-4-Ethyl-9-[1(S)-methyl-3-acetoxypropyl]-3,8,10-trimethyl-2-oxo-1,11-dihydrodipyrrinone (8). A mixture of 1.36 g (4.5 mmol) of alcohol 17, 10 mL of dry pyridine, and 4.25 mL (45 mmol) of acetic anhydride was stirred for 16h. It was diluted with 150 mL of CH₂Cl₂, washed carefully with 5% NaHCO₃ (2×100 mL), H₂O (2×100 mL) and dried over anhydrous Na₂SO₄. After filtering, the solvent was removed under vacuum. The resulting solid was recrystallized from CHCl₃/CH₃OH to afford 1.37 g (88%) of bright yellow dipyrrinone 8. It had mp

146-148°C; $[\alpha]_D^{20}$ +82.2° (*c* 0.7, CHCl₃); ¹H-NMR: δ 1.18 (3H, t, J=7.6 Hz), 1.29 (3H, d, J=7.1 Hz), 1.94 (3H, s), 1.90-1.99 (2H, m), 2.03 (3H, s), 2.15 (3H, s), 2.44 (3H, s), 2.55 (2H, q, J=7.6 Hz), 2.85-2.93 (1H, m), 3.86-3.98, 4.00-4.09 (2 × 1H, 2 × m), 6.13 (1H, s), 10.32 (1H, br.s), 11.31 (1H, br.s) ppm; and ¹³C-NMR: δ 8.55, 10.30, 12.57, 15.04, 17.95, 20.84, 21.01, 27.38, 35.11, 63.57, 100.94, 122.31, 122.38, 123.39, 124.21, 127.10, 130.98, 148.34, 171.10, 174.10 ppm.

Anal. Calcd. for C₂₀H₂₈N₂O₃ (344.4): C, 69.74; H, 8.19; N, 8.13. Found: C, 69.82; H, 8.24; N, 7.78.

(+)-4-Ethyl-9-[1(*S*)-methyl-3-acetamidopropyl]-3,8,10-trimethyl-2-oxo-1,11-dihydrodipyrrinone (9). A mixture of 655 mg (2 mmol) of azide 20, 20 mL of THF, 788 mg (3 mmol) of triphenyl phosphine, and 1.2 mL of H₂O was heated at reflux for 3h; then the solvents were evaporated under vacuum. The residual water was co-evaporated with 3×20 mL of dry benzene. The residue was diluted with 20 mL of dry CH₂Cl₂ and to it was added 2 mL of acetic acid, 6 mg of DMAP, and 1.24 g (6 mmol) dicyclohexylcarbodiimide. The mixture was stirred for 45 min., concentrated under vacuum to 10 mL, and filtered to remove dicyclohexylurea. The filtrate was purified by radial chromatography (eluent: 3-4.5% CH₃OH in CH₂Cl₂) and the chromatographically pure product was crystallized from CH₂Cl₂-dry Et₂O to afford 640 mg (93%) of acetamide 9. It had 196-198°C; $[\alpha]_D^{20}$ +44.5° (*c* 0.3, CHCl₃); ¹H-NMR: δ 1.17 (3H, t, J=7.6 Hz), 1.27 (3H, d, J=7.1 Hz), 1.81-1.89 (2H, m), 1.88 (3H, s), 1.94 (3H, s), 2.16 (3H, s), 2.44 (3H, s), 2.55 (2H, q, J=7.6 Hz), 2.74-2.83 (1H, m), 3.02-3.11 (1H, m), 3.25-3.34 (1H, m), 5.34 (1H, br.t), 6.13 (1H, s), 10.32 (1H, br.s), 11.30 (1H, br.s) ppm; and ¹³C-NMR: δ 8.52, 10.35, 12.57, 15.03, 17.90, 20.99, 23.26, 28.99, 35.83, 38.97, 100.74, 122.36, 122.41, 123.66, 123.92, 127.16, 130.75, 148.35, 169.92, 174.06 ppm.

Anal. Calcd. for $C_{20}H_{29}N_3O_2$ (343.5): C, 69.94; H, 8.51; N, 12.23. Found: C, 70.24; H, 8.55; N, 12.22.

Mesobiliverdin-XIII α analogs 4-6. General procedure. A mixture of 1 mmol of dipyrrinone 7-9, 615 mg (2.5 mmol) of *p*-chloranil, 220 mL of CH₂Cl₂, and 11 mL of formic acid (97%) was heated at reflux for 24h. The volume of the mixture was reduced by distillation to one half, and reflux was continued for 6h. Then the mixture was chilled overnight at -20°C. The separated solid was filtered, and the cold filtrate was carefully neutralized with saturated aqueous NaHCO₃. The organic layer was washed consecutively with 2 × 100 mL of 1 M NaOH and 4 × 200 mL of H₂O, then dried over anhydrous Na₂SO₄. After removing the solvent under vacuum, the crude product was purified by radial chromatography eluting with 1.5-2% CH₃OH in CH₂Cl₂ for 4 or 5, and 3-5% CH₃OH in CH₂Cl₂ for 6 to afford bright blue mesobiliverdin-XIII α analogs (4-6).

3,17-Diethyl-8,12-bis-[1(S)-methylpropyl]-2,7,13,18-tetramethyl-(21*H***,24***H***)-bilin-1,19-dione (4). This verdin was synthesized from dipyrrinone 7 in 88% yield. It had mp 267-269°C; ¹H-NMR: δ 0.88 (6H, t, J=7.3 Hz), 1.22 (6H, t, J=7.6 Hz), 1.35 (6H, d, J=7.1 Hz), 1.67-1.77 (4H, m), 1.82 (6H, s), 2.11 (6H, s), 2.53 (4H, q, J=7.6 Hz), 2.77-2.87 (2H, m), 5.99 (2H, s), 6.94 (1H, s), 8.00 (2H, br.s), 9.7 (1H, very br.s) ppm; and ¹³C-NMR: δ 8.44, 10.33, 12.81, 14.48, 17.94, 21.32, 30.43, 33.46, 96.32, 115.58, 127.04, 127.93, 139.31, 140.65, 143.70, 146.79, 149.78, 172.00 ppm.**

Anal. Calcd. for $C_{35}H_{46}N_4O_2 \cdot \frac{1}{2}$ CH3OH (570.8):C, 74.70; H, 8.48; N, 9.82.Found:C, 75.08; H, 8.24; N, 10.01.

3,17-Diethyl-8,12-bis-[3-acetoxy-1(S)-methylpropyl]-2,7,13,18-tetramethyl-(21*H***,24***H***)-bilin-1,19-dione (5). This verdin was synthesized from dipyrrinone 8** in 69% yield. It had mp 204-207°C; ¹H-NMR: δ 1.22 (6H, t, J=7.5 Hz), 1.38 (6H, d, J=7.0 Hz), 1.81 (6H, s), 2.02 (6H, s), 2.01-2.10 (4H, m), 2.12 (6H, s), 2.51 (4H, q, J=7.5 Hz), 3.03-3.13 (2H, m), 3.87-3.96, 4.06-4.14 (4H, two m), 5.96 (2H, s), 6.85 (1H, s), 8.01 (2H, br.s), 10.0 (1H, very br.s) ppm; and ¹³C-NMR: δ 8.25, 10.24, 14.39, 17.85, 20.88, 21.37, 28.02, 35.83, 63.01, 95.93, 114.51, 127.18, 128.21, 139.77, 140.41, 142.02, 146.80, 150.11, 170.76, 172.01 ppm.

Anal. Calcd. for $C_{39}H_{50}N_4O_6$ (670.8): C, 69.82; H, 7.51; N, 8.35. Found: C, 69.86; H, 7.49; N, 8.40.

3,17-Diethyl-8,12-bis-[3-acetamido-1(S)-methylpropyl]-2,7,13,18-tetramethyl-(21*H***,24***H***)-bilin-1,19-dione (6). This verdin was synthesized from dipyrrinone 9 in 56% yield. It had mp 221-223°C; ¹H-NMR: δ 1.20 (6H, t, J=7.6 Hz), 1.36 (6H, d, J=7.1 Hz), 1.80 (6H, s), 1.86 (6H, s), 1.88-1.96 (4H, m), 2.11 (6H, s), 2.51 (4H, q, J=7.6 Hz), 2.94-3.05 (4H, m), 3.29-3.38 (2H, m), 5.96 (2H, s), 6.41 (2H, br.s), 6.88 (1H, s), 8.33 (2H, br.s), 9.9 (1H, very br.s) ppm; and ¹³C-NMR: δ 8.27, 10.31, 14.42, 17.83, 21.73, 23.06, 29.81, 36.72, 38.89, 96.25, 114.92, 127.02, 128.19, 139.78, 140.32, 142.65, 146.89, 150.04, 170.41, 172.37 ppm.**

Mesobilirubin-XIIIa analogs 1-3. General procedure. To a solution of 0.25 mmol of mesobiliverdin-XIIIa analog 4-6 in 50 mL of dry deoxygenated THF, cooled in an ice bath, was added 945 mg (25 mmol) sodium borohydride followed by dropwise addition of 25 mL of dry CH_3OH over 15 min. under N_2 . After stirring 30-45 min., the mixture was diluted with 150 mL of degassed ice water and acidified with 10% aq. HCl, added very slowly under N_2 while cooling with ice bath. The mixture was diluted further with 100 mL of H_2O , and the product was extracted into $CHCl_3$ (3 × 100 mL). The combined extracts were washed with H_2O (3 × 100 mL) until neutral, dried over anhydrous Na_2SO_4 , and filtered. The solvent was removed under vacuum (<30°C), and the resulting crude product was purified by radial chromatography, eluting with 1.5-3% CH_3OH in CH_2Cl_2 for 1 and 2 or 3.5-5% CH_3OH in CH_2Cl_2 for 3. After removing the solvent, the bright yellow fractions were recrystallized from a minimal volume of chloroform and methanol (added dropwise).

(-)-3,17-Diethyl-8,12-bis-[1(S)-methylpropyl]-2,7,13,18-tetramethyl-(10H,21H,23H,24H)-bilin-1,19-dione (1). This rubin was obtained from verdin 4 in 89% yield. It had mp 246-249°C; $[\alpha]_D^{20}$ - 1490° (*c* 8.4 × 10⁻³, CHCl₃). ¹H-NMR: **\delta** 0.83 (6H, t, J=7.3 Hz), 0.99 (6H, t, J=7.5 Hz), 1.30 (6H, d, J=7.1 Hz), 1.40 (6H, s), 1.62-1.72 (4H, m), 2.15 (6H, s), 2.31 (4H, q, J=7.5 Hz), 2.76-2.85 (2H, m), 4.12 (2H, s), 5.91 (2H, s), 10.17 (2H, br.s), 10.77 (2H, br.s) ppm; and ¹³C-NMR: **\delta** 7.54, 11.08, 12.88, 14.74, 17.77, 20.69, 23.18, 29.60, 33.10, 99.98, 122.13, 123.26, 124.10, 124.17, 128.55, 131.01, 146.72, 174.06 ppm.

Anal. Calcd. for $C_{35}H_{48}N_4O_2 \cdot 1.5$ CH₃OH (604.8): C, 72.48; H, 9.00; N, 9.26. Found: C, 72.55; H, 8.31; N, 9.32.

(-)-3,17-Diethyl-8,12-bis-[3-acetoxy-1(S)-methylpropyl]-2,7,13,18-tetramethyl-(10H,21H,23H,24H)-bilin-1,19-dione (2). This rubin was synthesized from verdin 5 in 67% yield. It had mp 251-253 °C (decomp.); $[\alpha]_D^{20}$ -1140° (c 4.4 × 10⁻³, CHCl₃); ¹H-NMR: δ 0.99 (6H, t, J=7.5 Hz), 1.35 (6H, d, J=7.1 Hz), 1.41 (6H, s), 1.94-2.03

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(4H, m), 2.02 (6H, s), 2.16 (6H, s), 2.32 (4H, q, J=7.5 Hz), 3.02-3.11 (2H, m), 3.88-3.97, 4.00-4.08 (4H, two m), 4.10 (2H, s), 5.90 (2H, s), 10.20 (2H, br.s), 10.74 (2H, br.s) ppm; and ¹³C-NMR: δ 7.55, 10.96, 14.72, 17.76, 20.98, 21.06, 22.98, 27.94, 35.12, 63.65, 99.81, 121.83, 122.74, 123.56, 124.41, 129.02, 130.44, 146.90, 170.96, 174.19 ppm.

Anal. Calcd. for $C_{39}H_{52}N_4O_6$ (672.8):C, 69.61; H, 7.79; N, 8.33.Found:C, 69.44; H, 7.75; N, 8.25.

(-)-3,17-Diethyl-8,12-bis-[3-acetamido-1(*S*)-methylpropyl]-2,7,13,18-tetramethyl-(10H,21H,23H,24H)bilin-1,19-dione (3). This rubin was synthesized from verdin 6 in 74% yield. It had mp 255-258°C (decomp.); $[\alpha]_D^{20}$ - 1690° (*c* 9.8 × 10⁻³, CHCl₃); ¹H-NMR: δ 1.03 (6H, t, J=7.6 Hz), 1.33 (6H, d, J=7.0 Hz), 1.52 (6H, s), 1.73 (6H, s), 1.81-1.94 (4H, m), 2.14 (6H, s), 2.35 (4H, q, J=7.6 Hz), 2.90-3.00 (4H, m), 3.27-3.36 (2H, m), 4.12 (2H, s), 5.70 (2H, br.s), 5.96 (2H, s), 10.16 (2H, br.s), 10.35 (2H, br.s) ppm; and ¹³C-NMR: δ 7.98, 11.05, 14.78, 17.80, 21.12, 22.89, 22.98, 29.87, 35.69, 38.93, 100.03, 121.86, 123.33, 123.88, 124.24, 129.99, 130.03, 147.24, 170.19, 174.35 ppm.

> Anal. Calcd. for $C_{39}H_{54}N_6O_4$ (670.9): C, 69.82; H, 8.11; N, 12.53. Found: C, 69.60; H, 7.83; N, 12.31.

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