Tetrahedron Vol. 44, No. 15, pp. 4679 to 4688, 1988
Printed in Great Britain

0040-4020/88 $3.00 + .00

CONFORMATION OF BILIRUBIN AMIDES FROM CIRCULAR DICHROISM SPECTROSCOPY

DAVID A. LIGHTNER* and MAN-HUA ZHANG

Department of Chemistry, University of Nevada

Reno, Nevada 89557-0020 USA

(*Received in USA* 19 *February* 1988)

Abstract. The first optically active amides of bilirubin-IXα with amino acids show bisignate circular dichroism Cotton effects (CEs) in the vicinity of the pigment's long wavelength UV-visible absorption, e.g., for the bis-amide with L-alanine methyl ester in acetonitrile $\Delta\epsilon^{max}_{405} = -47$, $\Delta\epsilon^{max}_{450} = +68$ and $\epsilon^{max}_{435} = 55,800$. In marked contrast, the diester from S-(-)-lactic acid ethyl ester exhibits $\Delta\epsilon^{max}_{400} = +1.0$, $\Delta\epsilon^{max}_{450} = -2.6$ and $\epsilon^{max}_{394} = 52,800$ in acetonitrile. The CEs of the mono-amides are generally smaller than those of the bis-amides; whereas, the CEs of mono-esters are generally larger than those of the diesters.

INTRODUCTION

The yellow-orange, cytotoxic pigment of jaundice (4*Z*,15*Z*)-bilirubin-IXα (BR-IX), is produced abundantly in mammals by catabolism of heme and transported to the liver for glucuronidation and excretion.[1] Its structure has long been of interest,[2] especially recently in connection with phototherapy for neonatal jaundice[3] and also because of its unusal hydrophobic properties.[4] The latter have been attributed to a 3-dimensional structure[5,6] in which the polar carboxylic acid and lactam functionalities are linked through *intra*molecular hydrogen bonding (Fig. 1).[7] And in phototherapy, which involves, *inter alia*, configurational isomerization at the C_4-C_5 or C_{15}-C_{16} carbon-carbon double bonds, the associated H-bond breaking yields isomeric pigments that can be excreted by the liver without resort to conjugation.[1,3]

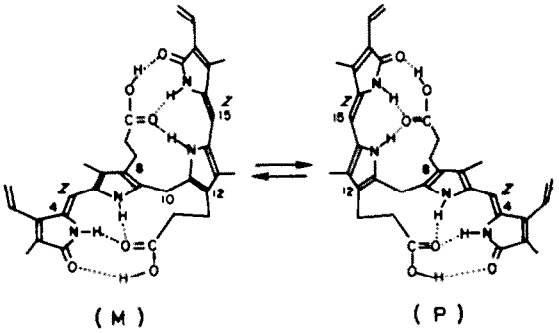

FIGURE 1. Interconverting, folded and intramolecularly hydrogen-bonded conformational enantiomers of (4*Z*,15*Z*)-bilirubin-IXα. Conformer M has the negative chirality orientation of the pyrromethenone long wavelength electric transition dipoles. Conformer P has the positive chirality orientation. (See ref. 12a)

BR-IX can fold into either of two enantiomeric conformations[1,5] that minimize steric repulsions[8] and are stabilized by intramolecular H-bonds yet interconvert with an activation energy of ~18 kcal/mole and a rate of 7.2 ±0.4 sec^{-1} (~58°C).[6a,b,9] As such, it can be viewed as a racemic mixture of conformational enantiomers, M ⇄ P (Fig. 1). In isotropic solvents, BR-IX solutions are optically inactive; however, in the presence of a chiral complexing agent, such as serum albumin[10,11] and optically active amines,[12,13] the solutions exhibit circular dichro-

ism (CD) for the intense bilirubin long wavelength UV-visible absorption near 450 nm. The origin of the induced optical activity can be seen in non-equal populations of M and P species in the complexes brought about by a first order asymmetric transformation[14] of the pigment.

Although there are many examples of proteins serving to induce CD of BR-IX[10,15] and a few examples of induced CD coming from BR-IX with optically active amines,[12,13,16] cyclodextrins[17] and micelles,[18] there are almost no examples of optically active bilirubin derivatives. The last include: (1) BR-IX glucuronides,[1] for which no CD spectra have been reported; (2) BR-IX linked covalently through its propionic acid groups to human serum albumins (HSA), $\Delta\epsilon^{max}_{500} \approx +6$, $\Delta\epsilon^{max}_{435} \approx -0.6$, $\Delta\epsilon^{max}_{470} \approx +3$ at pH 4.5-7.5;[19] (3) the δ-pigment,[20] $\Delta\epsilon^{max}_{368} \approx +3.1$, $\Delta\epsilon^{max}_{412} \approx -2.4$, $\Delta\epsilon^{max}_{450}$ +9.9 in pH 7.3 buffer;[21] (4) the *exo*-vinyl adducts of BR-IX with N-acetyl-L-cysteine and glutathione,[22] for which CD are not yet reported;[23] and (5) BR-IX esters made from *S*-(+)-2-butanol.[24] In the current investigation we report on the preparation and CD of the mono-amides and esters (as unseparated mixtures of C-8 and C-12 isomers; however, the stereochemical arguments of this work do not depend on differences in the ordering of the β-substituents on the distal lactam rings, and as noted previously,[12,17,18a] the 8- and 12-isomers are expected to give essentially the same CD signs and magnitudes) of BR-IX from *S*-(+)-alanine methyl ester (BR-IX MAA), *S*-(+)-2-butylamine (BR-IX MBA), *S*-(-)-lactic acid ethyl ester (BR-IX MLE) and *S*-(+)-2-butanol (BR-IX MBE), as well as the corresponding bis-amides and diesters: BR-IX BAA, BR-IX BBA, BR-IX DLE and BR-IX DBE, respectively. In each example the pigment is optically active by virtue of remote attachment of a chiral group, as in bilirubin glucuronides (ester) and in the δ-pigment and covalently bound HSA-bilirubin (ester or amide).

RESULTS AND DISCUSSION

Synthesis. The amides of this work were prepared by coupling the chiral amine to BR-IX using diphenylphosphoryl azide (Shioiri procedure[25]) as outlined earlier,[26] except dimethylsulfoxide was used as solvent. This modification was more suitable for pigments whose solubility in dimethylformamide is low. The esters were prepared from the *p*-toluenesulfonate of ethyl lactate and the methanesulfonate of 2-butanol using BR-IX bis-tetra-*n*-butylammonium salt.[24,27] The reaction proceeds via an S_N2 inversion of configuration of the alcohol component; so, the alcohol components of the pigment esters have the *R*-configuration and not the *S*-configuration of the starting alcohol.

Circular Dichroism and Conformation. The amide derivatives of this work exhibit bisignate CD Cotton effects (CEs) centered near the long wavelength UV-vis absorption of the pigment (summarized in Tables 1 and 2). The CE intensities are generally quite intense for the amino acid derivative; whereas, for the corresponding alkyl amine CE magnitudes are generally weaker, and the signed progression of the CEs is opposite. Several other interesting points emerge from the data of Tables 1 and 2: (1) High dielectric solvents that can serve only as H-receptors in H-bonding (Me_2SO) show significantly reduced CEs, but H-donor solvents in H-bonding (MeOH) show unexpectedly large CEs, comparable to or even larger than the CEs in low dielectric non-polar solvents (*cf.* C_6H_6 and CH_2Cl_2). (2) The bis-amides tend to exhibit larger CEs than do the mono-amides. Moreover, dilution has little or no effect on the CD, as expected from an intrinsic property of the molecule and one not associated with self-association.

The CE magnitudes of Table 1 would be considered surprisingly large for inherently symmetric chromophores with remote chiral perturbation,[28] e.g., optically active pyrromethenone esters and amides (xanthobilirubic acid (*R*)-α-phenethylamide: $\Delta\epsilon^{max}_{415} = -1.3$ for the monomer in Me_2SO and $\Delta\epsilon^{max}_{402} = -3.1$, $\Delta\epsilon^{max}_{446} = +1.3$ in $CHCl_3$ where intermolecularly H-bonded dimers form).[29] The data suggest a different mechanism for the origin of the optical activity. In fact, both the bisignate nature and large CEs of the alanyl amides are characteristic of bichromophoric molecules with large (π–π^*) transition moments and well-defined structures having little interchromophoric p-orbital overlap.[30] They are a clue to the pigment's 3-dimensional structure.

Intense bisignate CDs are also characteristic of bilirubin-protein heteroassociation complexes[10,11] and amine salts,[12] where the pigment is thought to assume a moderately well-defined folded, chiral conformation akin to either of those of Fig. 1. The tendency of BR-IX to adopt

Table 1. Circular Dichroism (CD) and Ultraviolet-Visible (UV) Spectral Data for 1.0-1.6 x 10^{-5} *M* Solutions of Bilirubin Mono- and Bis-Amides Formed from *S*-(+)-Alanine Methyl Ester[a]

		CD			UV	
Amide	Solvent[b]	$\Delta\epsilon_{max}(\lambda_1)$	λ_2 at $\Delta\epsilon$=0	$\Delta\epsilon_{max}(\lambda_3)$	ϵ_{max}	(λ, nm)
Mono	C_6H_6	-20.2 (402)	425	+27.5 (452)	49,000	(446)
Di		-40.0 (401)	423	+57.9 (450)	56,600	(443)
Mono	CH_2Cl_2	-11.9 (401)	423	+17.5 (448)	49,000	(446)
Di		-19.9 (395)	423	+34.7 (444)	60,000	(442)
Mono	Me_2CO	-33.3 (403)	423	+49.7 (451)	46,500	(440)
Di		-49.5 (395)	417	+81.9 (445)	59,000	(442)
Mono	MeCN	-26.5 (402)	421	+40.7 (450)	50,000	(438)
Di		-54.3 (395)	415	+83.8 (442)	58,600	(437)
Mono	MeOH	-13.6 (401)	423	+24.0 (452)	46,500	(446)
Di		-41.4 (403)	423	+60.2 (452)	57,100	(443)
Mono	Me_2SO	- 2.4 (405)	424	+ 6.0 (458)	50,200	(453)
Di		- 2.3 (399)	417	+11.1 (452)	58,900	(451)

[a] Prepared by Shioiri coupling (refs. 25,26) of BR-IX with L-alanine methyl ester. Spectra were run at 21°C within 0.5 hrs. of solution preparation using the corresponding amides of D,L-alanine methyl ester as the ($\Delta\epsilon$ = 0) baseline.

[b] C_6H_6 = benzene, CH_2Cl_2 = dichloromethane, Me_2CO = acetone, MeCN = acetonitrile, Me_2SO = dimethylsulfoxide.

[c] At 2.5 x 10^{-6} *M* the values are: (Mono) $\Delta\epsilon_{405}^{max}$ = -32.8, $\Delta\epsilon_{455}^{max}$ = +42.1, $\Delta\epsilon$ = 0 at 427 nm, ϵ_{435}^{max} = 55,800; (Di) $\Delta\epsilon_{400}^{max}$ = -44.8, $\Delta\epsilon_{450}^{max}$ = +66.0, $\Delta\epsilon$ = 0 at 421 nm ϵ_{435}^{max} = 52,000.

Table 2. Circular Dichroism (CD) and Ultraviolet-Visible (UV) Spectral Data for 1.2-2.5 x 10^{-5} *M* Solutions of Bilirubin-IXα Mono- and Bis-Amides[a] of *S*-(+)-2-Butylamine at 21°C.

		CD			UV	
Amide	Solvent[b]	$\Delta\epsilon_{max}(\lambda_1)$	λ_2 at $\Delta\epsilon$=0	$\Delta\epsilon_{max}(\lambda_3)$	ϵ_{max}	(λ, nm)
Mono	C_6H_6	+ 2.0 (415)	439	- 4.0 (470)	66,900	(453)
Di		+ 1.2 (405)	424	-16.3 (455)	61,200	(453)
Mono	CH_2Cl_2	+ 2.9 (410)	429	- 7.0 (460)	57,100	(448)
Di		+ 3.1 (410)	427	- 8.8 (460)	63,700	(448)
Mono	Me_2CO	« 0.1	---	- 2.6 (460)	64,400	(443)
Di		+ 5.1 (405)	426	-12.4 (455)	55,900	(440)
Mono	MeCN	« 0.1	---	« 0.1	55,600	(445)
Di		+ 6.2 (405)	420	-14.7 (455)	55,500	(435)
Mono	MeOH	+ 5.7 (415)	435	- 7.6 (465)	50,900	(447)
Di		+13.6 (405)	423	-27.3 (455)	52,600	(446)
Mono	Me_2SO	« 0.1	---	- 0.9 (465)	65,600	(453)
Di		+ 2.0 (410)	430	- 3.4 (460)	52,300	(450)

[a] Prepared by the procedure of Shioiri *et al.* (refs. 25,26). Spectra were run within 0.5 hrs. of solution preparation using the corresponding amides of racemic 2-aminobutane as the ($\Delta\epsilon$ = 0) baseline.

[b] C_6H_6 = benzene, CH_2Cl_2 = dichloromethane, Me_2CO = acetone, MeCN = acetonitrile, Me_2SO = dimethylsulfoxide.

[c] With added urea (0.18 *M*), the CD values are reduced to $\Delta\epsilon_{max}$ = +12.7 (410), $\Delta\epsilon$ = 0 at 430 nm, $\Delta\epsilon_{max}$ = -22.9 (457), UV: ϵ = 51,300 (446 nm).

a folded conformation is governed by factors other than intramolecular H-bonding,[8] but H-bonding sharply lowers the conformational global energy minimum, adding a powerful stabilization. Even partial H-bonding can be beneficial, as with mono-esters[31] and amides,[26] or when the propionic acid groups are deprotonated, as in the bis-isopropylammonium[32] and bis-tetra-*n*-alkylammonium salts.[33] So when the amides of BR-IX are formed from primary amines, one might view conformational stabilization as taking place through a propionamide N-H (Fig. 2) in place of the propionic acid O-H (Fig. 1). Since the amide N-H hydrogen is less acidic than the acid O-H, the effectiveness of H-bonding is probably lessened, but such secondary amide derivatives

of bilirubin offer a considerably greater potential for stabilizing conformations than do esters or tertiary amides.

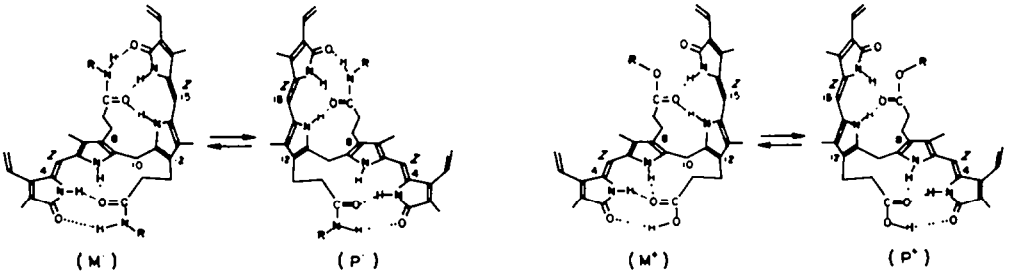

FIGURE 2. Interconverting, intramolecularly hydrogen-bonded enantiomeric conformations of BR-IX (left) bis-amides from 1° amines and (right) mono-esters. (Only the C_8 mono-ester is shown.) When the R group contains a chiral center, as in the amides and esters of this work, the interconverting structures are conformational diastereomers. The folded pigment conformations appear to be energy-minimum structures even without the H-bonding (ref. 8).

The mono- and bis-amides (BR-IX MAA, BR-IX MBA, BR-IX BAA, BR-IX BBA) may thus be viewed as pairs of interconverting diastereomers (M' P', Fig. 2) that have an enantiomeric disposition of the two pyrromethenone chromophores. Since diastereomers typically have $\Delta G_f^\circ(M') \neq \Delta G_f^\circ(P')$, the concentration of M' will not be equal to that of P', and the solutions will exhibit an optical activity characteristic of the predominant diastereomer. Large differences in diastereomer concentration are expected to yield large CEs, with values for a pure diastereomer approaching a rough theoretical limit,[12a] $|\Delta\epsilon| \simeq 260\ M^{-1}\ cm^{-1}$. However, the factors that affect the M' ⇌ P' equilibrium so as to generate the significantly larger CEs of the alanine amides (Table 1) *vs.* the *sec*-butyl amides (Table 2) are unclear. It may be noted that large CEs are also observed for the amides of L-tryptophan methyl ester.[34] Here and with the alanine amides the large CEs probably reflect a greater stabilization of one diastereomer through a stronger dipole-dipole interaction between the pyrromethenone and the amino acid, akin to that suggested to account for biliverdinoid (amino acid) amide CDs.[35] Although the amides of Tables 1 and 2 belong to the same (amine) absolute configuration series (*S*), the inversion of the signed order of the CEs is unexplained.

Like the mono-amides, mono-esters also retain partial intramolecular H-bonding, here through the underivatized propionic acid carboxylic acid group (Fig. 2).[27,31] And like its amide analog, the mono-ester from L-lactic acid ethyl ester exhibits bisignate CD CEs for the pigment's long wavelength absorption (Table 3). But unlike the L-alanyl mono-amide, the mono-ester CEs are generally weaker, tending to be largest in solvents that do not interfere with H-bonding. Furthermore, whatever influence the amino acid's carbomethoxy group might have on the amide CDs (Table 1 *vs.* Table 2), a carboethoxy or carbomethoxy group[36] apparently does not assume a similar importance to the CDs of the lactic acid esters, *cf.* the counterpart *sec*-butyl esters (Table 4). In fact the ester data of Tables 3 and 4 are very similar, and the signed order of the CEs is not reversed.

Unlike the bis-amides, the diester CEs are considerably weaker than those of the corresponding esters. Although diesters may assume the folded conformations of Figs. 1 and 2,[8] they do not achieve this with the added benefit of full H-bonding stabilization. In Me_2SO, H-bonding to solvent further destabilizes intramolecular H-bonding,[6c] and in non-polar solvents the pigment tends to dimerize through intermolecular pyrromethenone-pyrromethenone H-bonds.[6a,37-39] Our understanding of the CD of diesters is correspondingly complicated. Since the diester CEs show a much greater concentration dependence than do mono-esters, mono-amides or bis-amides over the range 10^{-5}-10^{-7} *M*, we assume that their CD may not be derived entirely from conformations as those in Fig. 2.

Table 3. Circular Dichroism (CD) and Ultraviolet-Visible (UV) Spectral Data for 1.7-3.9 x 10^{-5} *M* Solutions of Bilirubin Mono- and Di-Esters formed from *S*-(-)-Lactic Acid Ethyl Ester[a]

Ester	Solvent[b]	CD $\Delta\epsilon_{max}(\lambda_1)$	CD λ_2 at $\Delta\epsilon$=0	CD $\Delta\epsilon_{max}(\lambda_2)$	UV ϵ_{max}	UV (λ, nm)
Mono	C_6H_6	+13.9 (405)	428	-18.1 (453)	50,600	(442)
Di		+ 2.1 (400)	420	- 3.5 (450)	52,300	(398)
Mono	CH_2Cl_2	+30.9 (405)	428	-43.1 (455)	58,200	(438)
Di		+ 2.1 (405)	417	- 3.9 (450)	57,400	(396)
Mono	Me_2CO	+10.3 (405)	428	-15.3 (453)	57,100	(436)
Di		+ 1.0 (400)	412	- 2.7 (450)	58,200	(402)
Mono	MeCN	+ 5.2 (405)	426	- 6.9 (455)	51,200	(415)
Di		+ 1.0 (400)	420	- 2.6 (450)	52,800	(394)
Mono	MeOH	+ 3.0 (403)	424	- 6.7 (453)	63,100	(450)
Di		« 0.1	---	- 3.7 (455)	53,200	(458)
Mono	Me_2SO	« 0.1	---	- 3.1 (455)	50,200	(452)
Di		« 0.1	---	- 4.3 (455)	57,000	(450)

[a] Prepared by S_N2 reaction of BR-IX bis-tetra-*n*-butylammonium salt with the *p*-toluenesulfonate of *S*-(-)-lactic acid ethyl ester. Spectra were run at 20°C within 0.5 hrs. of solution preparation using the corresponding esters of racemic lactic acid ethyl ester as the ($\Delta\epsilon$ = 0) baseline.

[b] C_6H_6 = benzene, CH_2Cl_2 = dichloromethane, MeCN = acetonitrile, MeOH = methanol, Me_2SO = dimethylsulfoxide.

Table 4. Circular Dichroism (CD) and Ultraviolet-Visible (UV) Spectral Data for 2-5 x 10^{-5} *M* Solutions of the Bilirubin-IXα Mono- and Di-esters formed from *S*-(+)-2-Butanol[a]

Ester	Solvent[b]	CD $\Delta\epsilon_{max}(\lambda_1)$	CD λ_2 at $\Delta\epsilon$=0	CD $\Delta\epsilon_{max}(\lambda_2)$	UV ϵ_{max}	UV (λ_{nm})
Mono	C_6H_6	+12.6 (405)	424	-21.2 (450)	48,000	(438)
Di		+ 1.1 (400)	424	- 1.5 (450)	34,500	(402)
Mono	CH_2Cl_2	+10.2 (405)	424	-15.7 (450)	54,300	(438)
Di		+ 1.3 (400)	421	- 1.7 (450)	33,700	(398)
Mono	Me_2CO	+ 8.2 (405)	423	-12.2 (450)	60,000	(438)
Di		+ 0.6 (400)	420	- 0.86(450)	34,500	(406)
Mono	MeCN	+ 3.4 (405)	422	- 5.2 (450)	35,500	(420)
Di		« 0.1	---	« 0.1	37,700	(400)
Mono	MeOH	+ 1.3 (400)	423	- 2.2 (450)	51,400	(448)
Di		+ 1.0 (410)	---	« 0.1	35,400	(450)
Mono	Me_2SO	« 0.1	---	« 0.1	56,800	(454)
Di		+ 1.4 (415)	---	« 0.1	32,400	(453)

[a] Prepared by S_N2 reaction of BR-IX bis-tetra-*n*-butylammonium salt with the methanesulfonate of *S*-(+)-2-butanol. Spectra were run at 20°C within 0.5 hrs. of solution preparation using the corresponding esters of racemic 2-butanol as the ($\Delta\epsilon$ = 0) baseline.

[b] C_6H_6 = benzene, CH_2Cl_2 = dichloromethane, MeCN = acetonitrile, MeOH = methanol, Me_2SO = dimethylsulfoxide.

[c] $\Delta\epsilon_{max}$ = +1.1 (500), $\Delta\epsilon$ = 0 at 480 nm.

Exciton coupling and Absolute Configuration. The situation with mono-esters, mono-amides and bis-amides, where the chiral perturber is covalently attached to the pigment may be seen as equivalent to that of the heteroassociation complexes of BR-IX with chiral agents such as serum albumin[10,11] and amines[12] that act as chiral complexing agents and also induce CD of the pigment. In either case, the bichromophoric pigment may be viewed as a molecular exciton[30] containing two pyrromethenone chromophores held in a chiral orientation, as in Figs. 1 and 2. Electrostatic interaction of the pyrromethenone electric dipole transition moments[40] leads to an exciton splitting of the molecular excited state resulting in two long wavelength electronic

transitions, one higher in energy and one lower in energy, with the separation dependent on the relative orientation and strength of the transition moments. According to theory,[30] the two exciton transitions always have oppositely-signed CD CEs, typically flanking the UV-vis transition(s),[12,30] as is observed (Fig. 3). The absolute configuration of the predominant diastereomer can be predicted from exciton coupling theory and a knowledge of the pyrromethenone long wavelength UV-visible transition moment.[12a] Thus, BR-IX MAA and BR-IX BAA are predicted to have an excess of the pigment with P handedness, whereas, BR-IX MBA, BR-IX BBA, BR-IX MLE and BR-IX MBE are predicted to have an excess of the pigment with M handedness. From the intensity of the CE and using the calculated CEs for P or M,[12a] we estimate a diastereomeric excess of ~13% for BR-IX MAA in MeCN and 26% for BR-IX BAA in MeCN. In contrast, BR-IX MBA shows essentially no diasteriomeric excess, and BR-IX BBA, BR-IX MLE and BR-IX MBE show only ~2% diastereomeric excess in MeCN solvent.

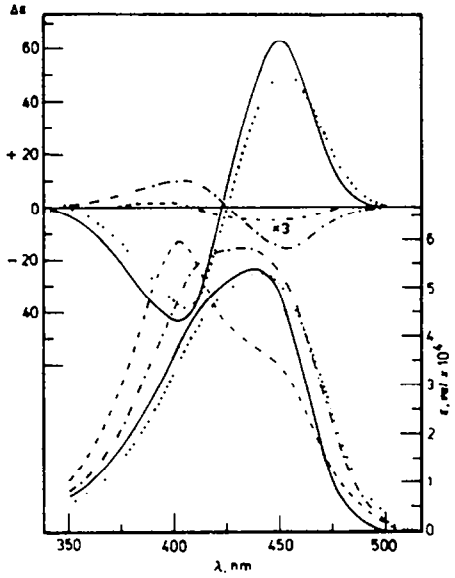

FIGURE 3. Circular dichroism and UV-vis spectra of 1.5 x 10^{-5} *M* solutions of BR-IX mono-amide of L-(+)-alanine methyl ester (———), the bis-amide (· · · · ·), BR-IX mono-ester from L-(-)-lactic acid ethyl ester(- - - -) and diester (—·—·) in MeCN run at 20°C. The mono-ester curve is magnified x 3. The S-shaped CD curves straddle the $\Delta\epsilon = 0$ line, which corresponds to the baseline run on samples made from D,L-alanine methyl ester and D,L-lactic acid ethyl ester. The UV-vis spectra show ϵ values increasing from the bottom of the Figure, the ϵ=0 baseline.

CONCLUDING COMMENTS

BR-IX tends to assume either of two folded, intramolecularly H-bonded conformations that interconvert by breaking and remaking all 6 H-bonds (Fig. 1) and may thus be seen as a racemic mixture. When the conformation-determining propionic acid carboxyl groups are derivatized as secondary amides, intramolecularly H-bonded conformations may still be retained (Fig. 2), but when the amide amine moiety possesses a chiral center, the erstwhile enantiomers become diastereomers, and the mixture is no longer racemic. More importantly, the equilibrium is no longer necessarily 1:1, [M'] ≠ [P'], and the optical activity associated with the mixture can be expected to show bisignate CD CEs associated with exciton coupling of the component pyrromethenone chromophores. Since the intrinsic CEs are typically very large for the enantiomeric conformers, moderate displacements from a 1:1 equilibrium will result in substantial CEs, as are observed for the bis-amides with L-alanine methyl ester. Even small displacements will still result in significant CEs, as are observed for the bis-amides with 2-aminobutane. Similarly, mono-amides and mono-esters, which still retain at least 50% of the intramolecular H-bonds through the underivatized propionic acid carboxyl group, can exhibit conformational diastereomerism when the amine or alcohol portion contains a chiral center. Here, too, conformations such as those of Fig. 2 appear to be essential in understanding the origin of the bisignate CDs.

Acknowledgement. We thank the National Institutes of Health (HD-17779) for generous support of this work. M-H. Zhang was a visiting scholar on leave from the Institute of Photographic Chemistry, Academia Sinica, Beijing.

EXPERIMENTAL

General: All circular dichroism (CD) spectra were run on a JASCO J-40 instrument equipped with photoelastic modulator, and all UV-vis spectra were run on a Cary 219 spectrophotometer. All NMR spectra were run on an IBM NR-80AF spectrometer in either d_6-dimethylsulfoxide (Aldrich, 99.9% d_6) or deuteriochloroform (99.9%, Aldrich). All IR spectra were obtained on a Perkin-Elmer model 599 instrument. Optical rotations, $[\alpha]_{589}$, were obtained on a Perkin-Elmer model 141 spectropolarimeter. Melting points were determined on a Mel-Temp capillary unit. Combustion microanalyses were obtained from Desert Analytics, Tucson, AZ. Analytical thin layer chromatography (TLC) was carried out using Analtech Uniplates (silica, 250μ); preparative TLC was carried out on Analtech Uniplates (silica, 500μ or 1000μ). Column chromatography was achieved using neutral aluminum oxide (activity II-III, Woelm). HPLC analyses were carried out on a Perkin-Elmer Series 3 liquid chromatograph equipped with an Altex 5μ ODS C-18 ultrasphere ion pairing column (25 cm) using 0.1 *M* di-*n*-octylamine acetate in 5% aq. methanol as eluent (flow rate 1.0 mL/min) and a column temperature of 23°C.[40] The retention times (min.) found were: BR-IX, 20; BR-IX BBA, 5; BR-IX MBA, 9; BR-IX BAA, 4.5; BR-IX MAA, 8; BR-IX BBE, 9; BR-IX MBE, 11; BR-IX BLE, 5.5; BR-IXα MLE, 9. Bilirubin-IXα was from Porphyrin Products, Logan, UT, and contained less than 5% of the IIIα and XIIIα isomers as determined by HPLC. *S*-(-)-Lactate acid ethyl ester, $[\alpha]_D^{20}$-12° (neat), diphenyl phosphoryl azide, methanesulfonyl chloride, *p*-toluenesulfonyl chloride, tetra-*n*-butylammonium hydroxide and triethylamine were from Aldrich. *S*-(+)-2-Aminobutane, $[\alpha]_D$+7.4° (neat) and *S*-(+)-2-butanol, $[\alpha]_D$+13.6° (neat) were from Norse Labs. S-(+)-alanine methyl ester hydrochloride was from Sigma. Pyridine was dried by distillation from K_2CO_3 and dimethylsulfoxide was dried by distillation from 3Å molecular sieves.

Bilirubin-IXα Mono- and Bis-amide of Alanine Methyl Ester (BR-IX MAA and BR-IX BAA: Bilirubin-IXα (29.2 mg, 0.05 mmoles), *S*-(+)-alanine methyl ester hydrochloride (14 mg, 0.1 mmoles), diphenylphosphoryl azide (11 mg, 0.1 mmoles) and triethylamine (20 mg, 0.2 mmoles) were stirred in 1 mL of dry Me_2SO in a 3 mL glass vial at room temperature in the dark under nitrogen for 1 h. An additional 14 mg of alanine methyl ester hydrochloride, 11 mg of diphenylphosphoryl azide and 10 mg of triethyl amine were then added, and the mixture was continuously stirred at room temperature in the dark under nitrogen for 3 h. HPLC analysis indicated that 40% of the mono-amide and 46% of the bis-amide were formed and that 14% of BR-IX was unreacted. Three milliliters of dichloromethane was then added and the solution was washed with cold water (3x1 mL) and dried (Na_2SO_4). Then the dichloromethane solution was applied to a column (10 cm long x 2 cm internal diameter) of neutral alumina and eluted with dichloromethane (200 mL) to remove unreacted azide and excess amines. BR-IX-BAA was then eluted with dichloromethane/methanol (20:1 v/v) (200 mL). After evaporation of the solvent, 5 mg of the bis-amide (15% yield) was obtained. The alumina column was then eluted with dichloromethane/methanol/ammonium hydroxide (30% aq.) (80:20:2 v/v/v) until an orange band of the mono-amide separated from a small amount of unreacted BR-IX, left at the top of the column. The alumina was extruded and the mono-amide band was excised and extracted to give 11 mg (30% yield) of BR-IX MAA. It had dec. >200°C; $[\alpha]_{589}^{20}$ = +420° (c 0.02, CH_2Cl_2); IR (KBr) ν: 3430, 3350, 2930, 1750, 1690, 1625, 1450, 1260, 1000, 710 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.20 (1.55) (d, 3H, CH_3 J = 7 Hz), 1.95-2.12 (12H, 4xCH_3), 2.6-2.78 (m, 8H, 4xCH_2), 3.51 (3.75) (s, 3H, CH_3), 4.02 (s, 2H, CH_2), 4.17 (4.26) (q, 1H, CH, J = 7 Hz), 5.24-6.78 (m, 8H, =CH), 9.23 (d, 1H, O=C-*NH*-, J = 7 Hz), 9.34 (s, 1H, pyrrole NH), 10.06 (9.91) (s, 1H, pyrrole NH), 10.69 (s, 1H, lactam NH), 10.79 (s, 1H, lactam NH) ppm.

Anal. Calcd for $C_{37}H_{43}N_5O_7$ (669.8): C, 66.35; H, 6.47; N, 10.46.
Found: C, 65.96; H, 6.51; N, 10.14.

The bis-amide had m.p. 137-140°C; $[\alpha]_{589}^{20}$ = +370° (c 0.02, CH_2Cl_2); IR (KBr) ν: 3430, 2930, 1750, 1680, 1660, 1640, 1550, 1460, 1260, 1220, 1175, 1000, 710 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.38 (d, 6H, 2xCH_3 J = 7 Hz), 1.73-1.97-2.10 (12H, 4xCH_3), 2.59-2.83 (m, 8H, 4xCH_2), 3.72 (s, 6H, 2xCH_3), 4.12 (s, 2H, CH_2), 4.66 (q, 2H, 2xCH, J = 7 Hz), 5.20-6.77 (m, 8H, =CH), 10.13 (d, 2H, O=C-*NH*), 10.40 (s, 1H, pyrrole), 10.70 (s, 1H, pyrrole NH), 11.03 (s, 1H lactam NH) ppm.

Anal. Calcd for $C_{41}H_{50}N_6O_8$ (754.9): C, 65.24; H, 6.68; N, 11.13.
Found: C, 64.88; H, 6.62; N, 10.75.

Bilirubin-IXα Mono-2-butyl Amide (BR-IX MBA): Bilirubin IXα (29.2 mg, 0.05 mmoles), *S*-(+)-2-aminobutane (7.3 mg, 0.1 mmoles), diphenylphosphoryl azide (11.6 mg, 0.1 mmoles) and triethylamine (10.1 mg, 0.1 mmoles) were stirred in 1 mL of dried Me_2SO in a 3 mL glass vial at room temperature under nitrogen in the dark for 1 h. Another 7.3 mg of 2-aminobutane and 11.6 mg of diphenylphosphoryl azide were then added and the mixture was stirred continuously at room temperature under nitrogen in the dark for 5 hrs. HPLC analysis indicated that 38% of the mono-amide and 44% of the bis-amide was formed and that 18% of BR-IXα was unreacted. Three milliliters of dichloromethane was then added. The solution was washed with cold water (3x1 mL) and dried over sodium sulfate. Then the dichloromethane solution was applied to a column (10 cm long x 2 cm internal diameter) of neutral alumina and eluted with dichloromethane (200 mL) to remove azide and excess amine. A small amount of BR-IX BBA was eluted with dichloromethane/-methanol (20:1 v/v, 200 mL). Elution was continued with dichloromethane/methanol/ammonium hydroxide (30% aq.) (80:20:2 v/v/v) until an orange band of the mono-amide was separated from a small band of unreacted BR-IX at the top of column. The alumina was then extruded from the column, and the band corresponding to BR-IX MBA was excised. The purified mono-amide was washed from the alumina with dichloromethane/methanol/glacial acetic acid (98:1:1 v/v/v). The acidic dichloromethane eluate was washed with water to pH=7, dried over sodium sulfate and evaporated at room temperature to give the mono-amide, 10 mg (27% yield) with mp. 180-3°C (dec.). (Careful deoxygenation of all of solvents and exclusion of light from the chromatography retarded product isomerization.) BR-IX MBA had $[\alpha]_{589}^{20}$ = -366° (CH_2Cl_2); IR (KBr) ν: 3410, 3340, 2980, 2920, 1680, 1645, 1615, 1445, 1250, 995 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.93 (t, 3H, CH_3 J = 6 Hz), 1.24 (d, 3H, CH_3 J = 6 Hz), 1.54 (q, 2H, CH_2, J = 6 Hz), 1.96-2.16 (12H, 4xCH_3),

2.32-2.79 (m, 8H, 4xCH_2), 3.44 (m, 1H, CH) 4.04 (s, 2H, CH_2), 5.27-6.78 (m, 8H, =CH), 8.76 (d, 0.5 H, O=C-*NH*-,*cis*, J = 6 Hz), 8.84 (d, 0.5 H, O=C-*NH*-,*trans*, J = 6 Hz), 9.39 (s, 1H, pyrrole NH), 10.26 (s, 1H, pyrrole NH), 10.71 (s, 1H, lactam NH), 10.82 (s, 1H, lactam NH) ppm.

Anal. Calcd for $C_{37}H_{45}N_5O_5$ (639.8): C, 69.46; H, 7.10; N, 10.95.
Found: C, 69.45; H, 6.70; N, 10.68.

Bilirubin-IXα Bis-2-butyl Amide (BR-IX BBA): Bilirubin-IXα (29.2 mg, 0.05 mmoles), *S*-(+)-2-aminobutane (36.7 mg, 0.5 mmoles), diphenylphosphoryl azide (58 mg. 0.5 mmoles) and triethylamine (50.5 mg. 0.5 mmoles) were stirred in 1 mL of dried Me_2SO in a 3 mL of glass vial at room temperature in the dark under nitrogen for 24 hrs. HPLC analysis indicated that the BR-IX was completely converted to the bis-amide. Three milliliters of dichloromethane was then added, and the solution was washed with cold water (5x1 mL), dried over sodium sulfate and evaporated to dryness. The residue was washed with diethyl ether (anhydrous) (5x5 mL) to extract the yellow-orange BR-IX BBA, 17.6 mg (50% yield), m.p. 204-206°C (dec). It had $[\alpha]_{589}^{20}$ = -512° (CH_2Cl_2); IR (KBr) ν: 3340, 2980, 2920, 1680, 1635, 1445, 1255, 1170, 995 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.93 (t, 6H, 2xCH_3, J = 6 Hz, 1.24 (d, 6H, 2xCH_3 J = 6 Hz) 1.50 (q, 4H, 2xCH_2, J = 6 Hz), 1.96-2.16 (12H, 4xCH_3), 2.32-2.79 (m, 8H, 4xCH_2) 3.41 (m, 2H, 2xCH), 4.02 (s, 2H, CH_2) 5.21-6.66 (m, 8H, =CH), 8.7 (d, 1H, O=C-*NH*-,*cis*, J = 6 Hz) 8.8 (d, 1H, O=C-*NH*-,*trans*, J = 6 Hz), 10.35 (s, 1H, pyrrole NH), 10.35 (s, 1H, lactam), 10.87 (s, 1H, pyrrole NH), 11.19 (s, 1H, lactam NH) ppm.

Anal. Calcd for $C_{41}H_{54}N_6O_4$ (694.9): C, 70.86; H, 7.83; N, 12.09.
Found: C, 70.56; H, 8.14; N, 12.18.

Bilirubin-IXα Mono- and Di-esters From *S*-(+)-2-Butanol (BR-IX MBE and BR-IX DBE): Bilirubin-IXα (29.2 mg, 0.05 mmoles) and 1% aq. tetra-*n*-butylammonium hydroxide (2.6 mL, 0.10 mmoles) were stirred in a glass vial at room temperature in dark under nitrogen for 30 min. The reaction mixture was then evaporated to dryness at 40°C. Then *S*-(+)-2-butanol methanesulfonate (30 mg, 0.2 mmoles) and 3 mL of dichloromethane were added to the residue of BR-IXα bis-tetra-*n*-butylammonium propionate salt, and the dichloromethane solution was heated at reflux for 14 h. HPLC analysis indicated that 17% of diester, 73% of monoester and 10% unknown impurity were formed. The dichloromethane solution was cooled, washed with water (3x1 mL), then dried over sodium sulfate. The dry dichloromethane solution was then applied to a column (10 cm long x2 cm internal diameter) of neutral alumina with dichloromethane as eluant. Unreacted methanesulfonate was eluted first. BR-IX DBE was eluted next with dichloromethane/methanol (20:1 v/v) and purified by preparative TLC on silica gel (1 mm layer) by developing the chromatogram with dichloromethane/methanol/glacial acetic acid (98:1:1 v/v/v). The purified diester (R_f ~0.3) was eluted from the silica gel with dichloromethane/methanol (20:1 v/v) to give ~2 mg (yield ~6%). To isolate the mono-ester, the alumina column was continuously eluted with dichloromethane/methanol/ammonium hydroxide (30% aq.) (80:20:2 v/v/v) until an orange band of BR-IX MBE was separated from a small band of unreacted BR-IX bis salt, which remained at the top of column. The alumina was then extruded from the column, and the band corresponding to the mono-ester was excised. The purified mono-ester was washed from the alumina with dichloromethane/methanol/glacial acetic acid (98:1:1 v/v/v) and the acidic dichloromethane eluate was washed with water (2x5 mL) and dried over sodium sulfate. The solvent was evaporated at room temperature to give BR-IX MBE, 14 mg (43% yield). (Pigment isomerization was retarded by excluding light.)

BR-IX MBE had mp 169-171°C; $[\alpha]_{589}^{20}$ = -692° (CH_3OH); IR (KBr) ν: 3400, 3340, 3250, 2940, 2910, 1725, 1695, 1655, 1625, 1450, 1360, 1250, 1170, 990, 690, cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.89 (t, 3H, CH_3, J = 6 Hz), 1.01 (d, 2H, CH_3, J = 6 Hz), 1.25 (m, 2H, CH_2, J = 6 Hz), 1.95-2.12 (12H, 4xCH_3), 2.59-2.77 (m, 8H, 4xCH_2), 3.97 (s, 2H, CH_2), 4.84 (m, H, CH), 5.42-6.80 (m, 8H, =CH), 9.27 (s, 1H, pyrrole NH), 9.30 (s, 1H, pyrrole NH), 10.70 (s, 1H, lactam NH), 10.80 (s, 1H, lactam NH) ppm.

Anal. Calcd for $C_{37}H_{44}N_4O_6 \cdot H_2O$ (658.8): C, 67.45; H, 7.04; N, 8.50.
Found: C, 67.76; H, 6.62; N, 8.08.

BR-IX DBE had mp 123-127°C; $[\alpha]_{589}^{20}$ = -995° (CH_2Cl_2); IR (KBr) ν: 3340, 2980, 2930, 1730, 1670, 1630, 1450, 1370, 1260, 1170, 990, 690 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.89 (t, 6H, 2xCH_3 J = 6 Hz), 1.20 (d, 6H, 2xCH_3 J = 6 Hz), 1.50 (q, 4H, 2xCH_2 J = 6 Hz), 1.76-1.98-2.09 (12H 4xCH_3), 2.31-2.81 (m, 8H 4xCH_2), 4.18 (s, 2H, CH_2), 4.88 (m, 2H, 2xCH), 5.32-6.54 (m, 8H, =CH), 10.10 (s, 1H, lactam NH), 10.11 (s, 1H, pyrrole NH), 10.45 (s, 1H, pyrrole NH), 11.18 (s, 1H, lactam NH) ppm.

Anal. Calcd for $C_{41}H_{52}N_4O_6$ (696.90): C, 70.66; H, 7.52; N, 8.04.
Found: C, 70.38; H, 7.22; N, 7.93.

Bilirubin-IXα Mono- and Di-esters from *S*-(-)-Lactic and Ethyl Ester (BR-IX DLE and BR-IX BLE): Bilirubin-IXα (29.2 mg, 0.05 mmoles) and 1% aq. tetra-*n*-butylammonium hydroxide (2.6 mL, 0.10 mmoles) were stirred in a glass vial at room temperature in dark under nitrogen for 30 min. The reaction mixture was then evaporated to dryness at 40°C. Then the tosylate of *S*-(-)-lactic acid ethyl ester (54.4 mg, 0.2 mmoles) and 3 mL of acetone were added to the residue of BR-IXα bis-tetra-*n*-butyl ammonium propionate salt, which was not isolated and purified, and the acetone solution was heated at reflux for 12 h. HPLC analysis indicate that 46% of di-ester, 44% of mono-ester, 10% of unreacted BR-IXα bis-salt were formed. The acetone was evaporated, and 3 mL of dichloromethane was then added, and the solution was washed with water (3x1 mL) then dried over sodium sulfate. The dry dichloromethane solution contained mono-, bis-ester and BR-IX bis salt, which could be separated and purified by column chromotography on alumina, followed by preparative TLC on silica gel as described above to give 7 mg of pure BR-IX DLE (18% yield). It had mp 78-82°C; $[\alpha]_{589}^{20}$ = -355° (CH_2Cl_2); IR (KBr) ν: 3420, 3270, 2930, 1750, 1700, 1665, 1635, 1450, 1260, 1000, 710 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (t, 6H, 2xCH_3 J = 7 Hz), 1.47 (d, 6H, 2xCH_3 J = 7 Hz), 1.73-2.08 (12H, 4xCH_3), 2.53-2.83 (m, 8H, 4xCH_2), 4.15 (s, 2H, CH_2), 4.20 (q, 4H, 2xCH_2, J = 7 Hz), 4.92 (q, 2H, 2xCH, J = 7 Hz), 5.2-6.72 (m, 8H, =CH), 10.11 (s,

1H, lactam NH), 10.14 (s, 1H, pyrrole NH), 10.44 (s, 1H, pyrrole NH), 11.15 (s, 1H, lactam) ppm.

Anal. Calcd for $C_{43}H_{52}N_4O_{10}$ (784.9): C, 65.80; H, 6.68; N, 7.14.
Found: C, 65.78; H, 6.81; N, 7.45.

The purified BR-IX MLE (10 mg) was isolated (29% yield). It had mp 110°-113°C; $[\alpha]_{589}^{20}$ = -1425° (CH_2Cl_2); IR (KBr) ν: 3400, 3000, 2930, 1750, 1680, 1660, 1640, 1460, 1270, 1100, 1000, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.23 (d, 3H, CH_3, J = 7 Hz), 1.47 (t, 3H, CH_3, J = 7 Hz), 1.97-2.13-2.43 (12H, 4xCH_3), 2.81 (m, 8H, 4xCH_2), 3.98 (s, 2H, CH_2), 4.02 (q, 2H, CH_2, J = 7 Hz), 4.93 (q, 1H, CH, J = 7 Hz), 5.27-6.79 (m, 8H, =CH), 8.04 (8.14) (s, 1H, pyrrole NH), 8.89 (8.89) (s, 1H, pyrrole NH), 9.10 (9.10) (s, 1H, lactam NH), 10.69 (10.78) (s, 1H, lactam NH) ppm.

Anal. Calcd for $C_{38}H_{44}N_4O_8$ (684.8): C, 66.65; H, 6.48; N, 8.18.
Found: C, 66.45; H, 6.80; N, 8.68.

S-(-)-Lactic Acid Ethyl Ester p-Toluenesulfonate:[42] Ethyl S-(-)-lactate (4.00 g, 0.034 moles) was dissolved in pyridine (40 mL) and cooled in an ice-bath to -5°C. p-Toluenesulfonyl chloride (8.00 g, 0.042 moles) was added in small portions over a period of 15 min, and the resulting light yellow solution was kept for 24 h. at -20°C. Small portions of ice (1 g, 2 g, 2 g) were then added, with a period of 15 min. between additions, insuring that the temperature remained near 0°C. The solution was then poured into ice water (100 mL) and extracted with chloroform (3x25 mL). The chloroform extracts were dried over anhydrous sodium sulfate and evaporated. The syrupy residue was taken up in hexane-dichloromethane (10:1, 80 mL) and crystallized by rapid cooling in a dry ice-acetone bath to yield 7g (75% yield) of the tosylate. Recrystallization from hexane-dichloromethane (10:1) at -20°C afforded colorless needles, 5 g (54% yield), mp 30-32°C; $[\alpha]_{589}^{25}$ = -49.5° (CH_2Cl_2); ^1H-NMR ($CDCl_3$) δ: 1.21 (t, 3H, CH_3) 1.50 (d, 3H, CH_3) 2.46 (s, 3H, CH_3) 4.10 (q, 2H, CH_2), 7.30 (d, 2H, Ar), 7.78 (d, 2H, Ar) ppm.

S-(+)-2-Butanol Methanesulfonate:[43] S-(+)-2-butyl alcohol (0.74 g, 0.01 moles) was dissolved in cold dichloromethane (10 mL) and cooled in an ice bath (0°C). Pyridine (1.62 mL, 0.02 moles) was then added, followed by the addition of methanesulfonyl chloride (1.72 g, 0.015 moles) in small portions with constant stirring at the ice bath (<5°C) for 3 hrs. The mixture was washed with cold hydrochloric acid (2 *N*, 5x5 mL) after adding 20 mL of cold dichloromethane, then in succession with 5 mL of cold water, 5% aq. sodium bicarbonate (3x5 mL) and cold water (3x5 mL). The organic layer was dried over anhydrous sodium sulfate, and the dichloromethane was evaporated at room temperature. The crude S-(+)-2-butanol methanesulfonate (1.2 g, 80% yield) was purified by column chromatography (2 x 15 cm internal diameter) on silica gel by elution with hexane-dichloromethane (6:4) to give 0.88 g of S-(+)-2-butanol methanesulfonate (62% yield) as a colorless oil. It had $[\alpha]_{589}^{20}$ = +14.5° (CH_3OH); ^1H-NMR ($CDCl_3$) δ: 0.98 (t, 3H, CH_3), 1.36 (d, 3H, CH_3), 1.64 (q, 2H, CH_2), 2.88 (s, 3H, CH_3), 4.68 (q, 1H, CH) ppm.

REFERENCES

1. D.A. Lightner and A.F. McDonagh, *Accounts Chem. Res. 17*, 417-424 (1984).

2. A.F. McDonagh in *The Porphyrins*, Vol. VI (D. Dolphin, ed.), Academic Press, New York (1979), pp 293-491.

3. A.F. McDonagh and D.A. Lightner, *Pediatrics 75*, 443-455 (1985).

4. R. Brodersen in *Bile Pigments and Jaundice* (J.D. Ostrow, ed.), Marcel Dekker, Inc., New York (1986), pp 157-181.

5. (a) R. Bonnett, J.E. Davies, M.B. Hursthouse and G.M. Sheldrick, *Proc. R. Soc. London Ser. B202*, 249-268 (1978).
   (b) G. LeBas, A. Allegret Y. Mauguen, C. DeRango and M. Bailly, *Acta Crystallogr. B36*, 3007-3011 (1980).

6. (a) For leading references see D. Kaplan and G. Navon, *Isr. J. Chem. 23*, 177-186 (1983).
   (b) G. Navon, S. Frank and D. Kaplan, *J. Chem. Soc. Perkin Trans. II*, 1145-1149 (1984).
   (c) D. Kaplan and G. Navon, *Biochem. J. 201*, 605-613 (1982).

7. D.A. Lightner in *Bilirubin, Vol I* (K.P.M. Heirwegh and S.B. Brown, eds.), CRC Press, Boca Raton, FL (1982), pp 1-58.

8. (a) H. Falk and N. Müller, *Monatsh. Chem. 112*, 1325-1322 (1981).
   (b) H. Falk and N. Müller, *Tetrahedron 39*, 1875-1885 (1983).
   (c) R.V. Person and D.A. Lightner, unpublished calculations.

9. P. Manitto and D. Monti, *J. Chem. Soc. Chem. Commun.* 122-123 (1976).

10. For leading references, see G. Blauer, *Isr. J. Chem. 23*, 201-209 (1983).

11. D.A. Lightner, M. Reisinger and G.L. Landen, *J. Biol Chem. 261*, 6034-6038 (1986).

12. (a) D.A. Lightner, J.K. Gawronski and W.M.D. Wijekoon, *J. Am. Chem. Soc. 109*, 6248-6259 (1987).
   (b) D.A. Lightner and J.Y. An, *Tetrahedron 43*, 4287-4296 (1987).

13. D. Marr-Leisy, K. Lahiri and P. Balaram, *Int. J. Peptide Protein Res. 25*, 290-296 (1985).

14. E.E. Turner and M.M. Harris, *Quart. Rev. Chem. Soc.*, 299-330 (1947).

15. G. Blauer, *Arch. Biochem. Biophys. 884*, 602-604 (1986).

16. That BR-IX can give an ICD in the presence of (+) or (-)-α-phenylethylamine in organic solvents has been cited by Blauer in ref. 10 and again in a discussion following a lecture by G. Blauer, and E. Lavie in *Protein-Ligand Interactions* (H. Sund and G. Blauer, eds.), pp 399-416, W. de Gruyter, NY (1975), for work of O. Mayer, Ph.D. thesis, Univ. Konstanz, (1979).

17. D.A. Lightner, J.K. Gawroński and K. Gawrońska, *J. Am. Chem. Soc. 107*, 2456-2461 (1985).

18. (a) For leading references, see M. Reisinger and D.A. Lightner, *J. Inclus. Phenom. 3*, 479-485 (1985).
(b) K.L. Puranam and P. Balaram, *Proc. Indian Acad. Sci. (Chem. Sci.) 98*, 453-468 (1987).

19. C.C. Kuenzle, N. Gitzelman-Cumarasamy and K.J. Wilson, *J. Biol. Chem. 251*, 801-807 (1976).

20. A.F. McDonagh, L.A. Palma, J.J. Lauff and T-W. Wu, *J. Clin. Invest. 74*, 763-770 (1984) and references therein.

21. M. Reisinger, T-W. Wu and D. A. Lightner, unpublished work.

22. P. Manitto and D. Monti, *Experientia 28*, 379-380 (1972).

23. W.M.D. Wijekoon and D.A. Lightner, in preparation.

24. D.A. Lightner, M-H. Zhang and F.R. Trull, *Tetrahedron Lett. 28*, 4033-4036 (1987).

25. T. Shioiri, K. Hinomiya and S. Yamada, *J. Am. Chem. Soc. 94*, 6203-6205 (1972).

26. D.A. Lightner, T.C. Adams and J-S. Ma, *Tetrahedron 21*, 4253-4260 (1984).

27. D.A. Lightner, F.R. Trull and M-H. Zhang, *Tetrahedron Lett. 28*, 1047-1055 (1987).

28. C.W. Deutsche, D.A. Lightner, R.W. Woody and A. Moscowitz, *Annu. Rev. Phys. Chem. 20*, 407-448 (1969).

29. D.A. Lightner, M. Reisinger and W.M.D. Wijekoon, *J. Org. Chem. 52*, 5391-5395 (1987).

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

31. D.A. Lightner and J-S. Ma, *Spectrosc. Lett. 17*, 317-327 (1984).

32. A. Mugnoli, P. Manitto and D. Monti, *Acta Crystallogr. Sec. C38*, 1287-1291 (1983).

33. D.A. Lightner, J-S. Ma and X-X. Wu, *Spectroscopy Lett. 19*, 311-320 (1986).

34. The mono-amide(s) of L-tryptophan methyl ester show CD CEs: $\Delta\epsilon^{max}_{410}$ -12.3, $\Delta\ \epsilon^{max}_{460}$ +20.8 (C_6H_6); $\Delta\epsilon^{max}_{402}$ -46.6, $\Delta\epsilon^{max}_{453}$ +93.3 (MeCN); $\Delta\epsilon^{max}_{400}$ -29.5, $\Delta\epsilon^{max}_{453}$ +49.5 (MeOH); and $\Delta\epsilon^{max}_{405}$ -19.0, $\Delta\epsilon^{max}_{460}$ +27.5 (Me_2SO).

35. K. Grubmayr and H. Falk, *Monatsh Chem. 118*, 837-843 (1987).

36. The esters of L-lactic acid methyl ester show CD CEs within 10% of the values of Table 3.

37. A.R. Holzwarth, E. Langer, H. Lehner and K. Schaffner, *Photochem. Photobiol. 32*, 17-26 (1980).

38. H. Falk, T. Schlederer and P. Wohlschann, *Monatsh. Chem. 112*, 199-207 (1981).

39. D.A. Lightner and F.R. Trull, *Spectroscopy Lett. 16*, 785-803 (1983).

40. (a) G. Blauer and G. Wagnière, *J. Am. Chem. Soc. 97*, 1949-1954 (1975).
(b) H. Falk, K. Grubmayr, G. Höllbacher, L. Hofer, A. Leodolter, F. Neufingerl and J. Ribó, *Monatsh Chem. 108*, 1113-1130 (1977).

41. A.F. McDonagh, L.A. Palma, F.R. Trull and D.A. Lightner, *J. Am. Chem. Soc. 104*, 6860-6867 (1982).

42. K.N. Slessor and B.D. Johnston, *Can. J. Chem. 57*, 233-235 (1979).

43. R.S. Bly and R.A. Mateer, *J. Org. Chem. 38*, 1518-1527 (1973).