Chiral Induction in Biliverdin Covalently Bound to Amino Acids

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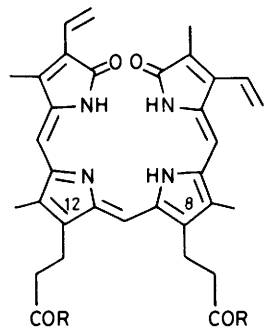
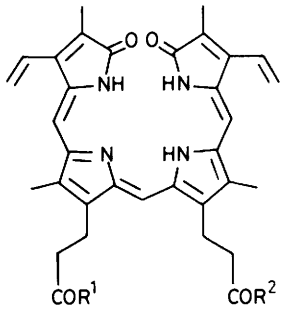
Amidation of the carboxy groups of biliverdin-IXα (**2**) with the appropriate (*S*)-amino acid methyl ester yields the optically active bis[(*S*)-amino acid] derivatives (**3**)—(**8**) which exhibit exceedingly strong Cotton effects in their visible and near-u.v. absorption bands. This is due to the influence of the chiral centre on the population of helical conformers of the bilatriene moiety across five bond lengths. The magnitude of the c.d. observed depends on the steric requirements of the amino acid and the polarity of the solvent. The chiral induction is interpreted in terms of intramolecular interactions of both side chains exerted from different sides of the bilatriene helix by hydrogen bonds. Both the donor and the acceptor sites of the amino acid residues are involved in the chiral association with the bilatriene backbone.

If the covalently bound apoproteins of biliproteins are cleaved off or denatured their tetrapyrrole moieties experience dramatic changes of their photophysical and photochemical properties. Thus, *in vivo*, the intramolecular non-bonded chromophore–protein interactions are believed to account for the conformation and rigidity of the light-sensitive chromophore so that reversible photochemical processes can take place.[1] While intermolecular interactions of open-chain tetrapyrroles with proteins[2] and other chiral agents[3–7] have been studied by several groups the investigations of intramolecular influences reported so far are mostly devoted to naturally occurring chromopeptides of high molecular masses.[8,9] The lack of a systematic study on this topic led us to the present investigation.

As model for the chromophore of biliproteins we have chosen biliverdin-IXα (**2**). Owing to the two carboxy groups in the side chains at C-8 and C-12, it can be bound covalently to the amino groups of amino acids and peptides. Biliverdins, like other bilatrienes in their (*Z*,*Z*,*Z*)-configuration, adopt a helical conformation;[3] the barrier of interconversion of the conformers of opposite chirality was estimated to be *ca.* 42 kJ mol^{-1}.[10] In chiral solvents[6] or in achiral solvents containing serum albumin[2] this equilibrium is shifted, giving rise to relatively large Cotton effects in the near-u.v. and visible absorption bands characteristic of the presence of an inherently chiral chromophore. Hence, c.d. should likewise serve as adequate means for the investigation of intramolecular induced conformational changes.

We now describe the influence of covalently bound neutral (*S*)-amino acid methyl esters on the conformation, *i.e.* helicity, of the bilatriene system.





(**1**) R = OMe
(**2**) R = OH
(**3**) R = (*S*)-Ala-OMe
(**4**) R = (*S*)-Phe-OMe
(**5**) R = (*S*)-Met-OMe
(**6**) R = (*S*)-Leu-OMe
(**7**) R = (*S*)-Trp-OMe
(**8**) R = (*S*)-Val-OMe
(**9**) R = (*S*)-OCHCOOCH$_2$CH$_3$ (CH$_3$)
(**10**) R = (*R*)-NHCHCH$_2$C$_6$H$_5$ (CH$_3$)

(**11**) R^1 = R^2 = OMe
(**12**) R^1 = OH, R^2 = OMe
(**13**) R^1 = R^2 = OH
(**14**) R^1 = R^2 = (*S*)-Ala-OMe
(**15**) R^1 = (*S*)-Ala-OMe, R^2 = OMe

Results and Discussion

Syntheses.—Biliverdin-IXα (**2**) and -XIIIα (**13**) were prepared from the corresponding isomer-free esters (**1**) and (**11**).[3,11] For the syntheses of the (*S*)-amino acid derivatives (**3**)—(**8**) biliverdin-IXα (**2**) was coupled with the appropriate optically pure (*S*)-amino acid methyl esters by means of *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodi-imide in tetrahydrofuran. The same procedure was applied for the preparation of compounds (**9**) and (**10**) using (*S*)-(−)-ethyl lactate and (*R*)-(−)-amphetamine, respectively.

Because of the unsymmetrical substitution pattern of biliverdin-IXα dimethyl ester (**1**) and the small differences in reactivity of the two methoxycarbonyl groups this compound is unsuited for partial saponification. To obtain a precursor for a mono-amino acid derivative we therefore took advantage of the higher symmetry of the XIIIα-isomer (**11**). After treatment with one equivalent of sodium hydroxide the monocarboxylic acid (**12**) and the dicarboxylic acid (**13**) were obtained. This mixture was coupled with (*S*)-(+)-alanine methyl ester as described above. Compounds (**14**) and (**15**) were separated *via* chromatography on silica gel.

The synthetic method applied proceeds without racemisation, as revealed by the absence of signals due to diastereoisomers in the ^{1}H n.m.r. spectra. Using optically impure starting materials we could easily detect diastereoisomers of compounds (**3**)—(**10**) and (**14**).

Because of the low volatility of the compounds prepared the molecular masses were determined by fast atom bombardment mass spectrometry (f.a.b.). The molecular masses of compounds (**3**), (**5**), and (**7**) were also determined by osmometry in chloroform solution. In all cases the masses of the monomers were obtained. Therefore self-association can be regarded to be $< 5\%$ even in 10^{-2}M solutions.

C.d. and Electronic Absorption Spectra.—The electronic absorption spectra of the IXα-derivatives (**3**)—(**10**) and the XIIIα-derivatives (**14**) and (**15**) (Table 1) do not differ

Table 1. U.v. and visible electronic absorption spectra [$\varepsilon_{max.}$/l mol^{-1} cm^{-1} ($\lambda_{max.}$/nm)] of optically active derivatives of biliverdin-IXα (**3**)—(**10**) and biliverdin-XIIIα (**14**) and (**15**) in benzene, chloroform, dichloromethane, ethanol, and tetrahydrofuran of *ca.* 10^{-4}M solutions at 20 °C

Compound	Benzene	Chloroform	Dichloromethane	Ethanol	Tetrahydrofuran
(**3**)	15 510 (653)	14 610 (658)	13 960 (658)	14 460 (665)	16 830 (645)
	51 100 (380)	53 450 (378)	52 400 (378)	51 600 (377)	54 470 (378)
(**4**)	14 110 (653)	14 540 (658)	13 800 (657)	13 180 (665)	15 450 (645)
	48 090 (381)	54 750 (378)	52 600 (377)	49 900 (377)	50 430 (378)
(**5**)	14 670 (655)	14 560 (660)	12 990 (660)	13 790 (664)	15 980 (645)
	51 780 (379)	55 760 (378)	51 630 (377)	51 750 (377)	53 090 (378)
(**6**)	14 900 (647)	12 920 (657)	13 650 (658)	13 050 (662)	15 060 (642)
	47 770 (380)	50 620 (379)	53 450 (378)	49 210 (377)	49 780 (378)
(**7**)	13 410 (672)	13 220 (675)	13 360 (672)	13 670 (667)	14 890 (650)
	45 640 (383)	48 360 (380)	48 380 (380)	49 800 (378)	51 070 (380)
(**8**)	13 640 (655)	13 170 (662)	14 200 (660)	13 470 (663)	15 260 (645)
	47 010 (379)	47 230 (377)	48 620 (378)	48 360 (376)	47 960 (377)
(**9**)	15 690 (657)	13 870 (660)	14 300 (660)	14 400 (663)	16 880 (644)
	48 310 (380)	51 330 (379)	56 360 (376)	53 530 (376)	53 500 (378)
(**10**)	14 910 (647)	13 350 (652)	13 070 (655)	14 090 (663)	16 020 (643)
	51 910 (381)	53 750 (378)	50 980 (378)	52 560 (377)	53 240 (378)
(**14**)	14 240 (643)	13 210 (650)	13 090 (652)	13 580 (657)	14 740 (639)
	38 050 (377)	37 840 (375)	38 440 (375)	40 160 (377)	39 100 (376)
(**15**)	15 280 (648)	15 240 (648)	15 560 (652)	14 640 (657)	15 660 (642)
	39 360 (378)	42 860 (377)	43 770 (378)	42 190 (376)	41 100 (377)

Table 2. C.d. data [$\Delta\varepsilon_{max.}$/l mol^{-1} cm^{-1} ($\lambda_{max.}$/nm)] of optically active derivatives of biliverdin-IXα (**3**)—(**10**) and biliverdin-XIIIα (**14**) and (**15**) in benzene, chloroform, dichloromethane, ethanol, and tetrahydrofuran of *ca.* 10^{-4}M solutions at 20 °C

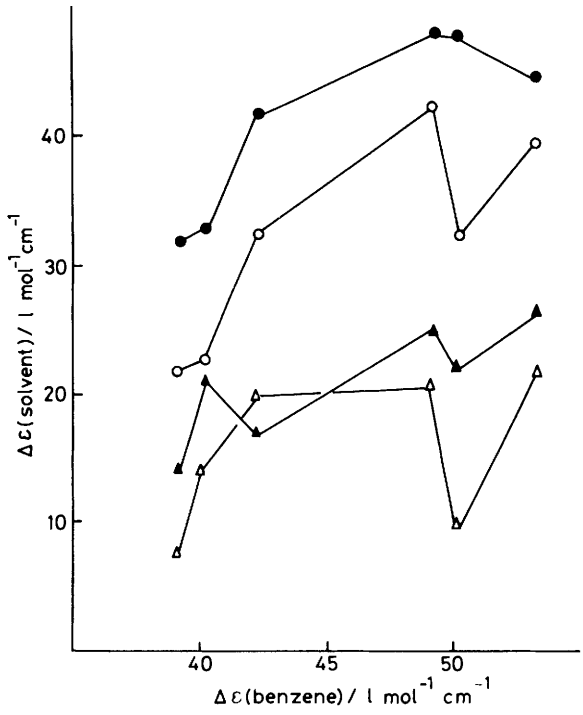
Compound	Benzene	Chloroform	Dichloromethane	Ethanol	Tetrahydrofuran
(**3**)	+39.0 (657)	+31.6 (657)	+21.9 (658)	+14.9 (663)	+7.5 (660)
	−56.9 (378)	−47.4 (376)	−33.0 (378)	−20.4 (377)	−13.6 (378)
(**4**)	+39.9 (662)	+33.4 (660)	+22.9 (658)	+21.4 (663)	+14.7 (662)
	−64.3 (378)	−52.7 (377)	−35.0 (376)	−31.7 (378)	−25.8 (378)
(**5**)	+42.0 (660)	+42.2 (658)	+33.4 (657)	+17.1 (660)	+20.3 (656)
	−63.9 (378)	−67.7 (376)	−52.2 (376)	−27.7 (377)	−33.9 (375)
(**6**)	+49.1 (660)	+48.5 (657)	+43.0 (658)	+24.8 (664)	+21.1 (660)
	−75.1 (378)	−76.7 (377)	−64.9 (377)	−35.4 (377)	−33.5 (377)
(**7**)	+50.2 (664)	+47.7 (663)	+33.3 (660)	+21.6 (662)	+10.2 (657)
	−81.7 (381)	−75.4 (378)	−52.4 (378)	−31.0 (382)	−17.5 (374)
(**8**)	+52.9 (660)	+45.2 (657)	+39.6 (655)	+27.3 (663)	+22.1 (660)
	−77.9 (378)	−67.8 (377)	−61.6 (378)	−38.2 (377)	−34.2 (377)
(**9**)	+1.2 (*ca.* 660)	+1.3 (*ca.* 655)	+1.1 (*ca.* 655)	+1.1 (*ca.* 650)	+0.3 (*ca.* 670)
	−1.4 (*ca.* 365)	−1.7 (*ca.* 360)	−1.3 (*ca.* 375)	−1.3 (*ca.* 375)	−0.4 (*ca.* 375)
(**10**)	+10.0 (666)	+1.5 (*ca.* 695)	+1.4 (*ca.* 685)	+8.3 (658)	+3.8 (662)
	−17.7 (383)	−1.7 (*ca.* 385)	−2.6 (*ca.* 385)	−15.4 (380)	−6.5 (378)
(**14**)	+36.6 (649)	+23.4 (647)	+19.7 (647)	+11.2 (656)	+6.7 (640)
	−45.5 (372)	−31.3 (372)	−23.2 (370)	−13.0 (377)	−9.1 (375)
(**15**)	+21.0 (650)	+11.7 (655)	+9.8 (650)	+6.8 (655)	+2.9 (655)
	−24.6 (372)	−12.5 (373)	−10.9 (370)	−7.9 (375)	−3.7 (372)

significantly from the spectra of the corresponding dimethyl esters (**1**) and (**11**).[4] Only the tryptophan derivative (**7**) shows a slight bathochromic shift of the long wavelength band by 6—18 nm except for ethanol solution.

The c.d. spectra of all optically active derivatives of biliverdin-IXα and -XIIIα (**3**)—(**10**) and (**14**) and (**15**), compiled in Table 2, have two main bands, one at λ *ca.* 660 nm and a second band at λ *ca.* 380 nm. Within 10 nm the c.d. maxima coincide with the corresponding maxima of the u.v. and visible electronic absorption bands (Table 1). Both bands are attributed to transitions of the bilatriene chromophore. Phenomenologically, the c.d. spectra resemble those obtained for biliverdin-IXα dimethyl ester (**1**) in chiral solvents.[3,4,6] The amino acid derivatives (**3**)—(**8**), (**14**), and (**15**) exhibit exceptionally large Cotton effects which are solvent dependent and decrease in the order: benzene > chloroform > dichloromethane > ethanol- > tetrahydrofuran (Table 2 and Figure). All spectral data obtained are independent of concentration within the range of 10^{-3}—10^{-6}M solutions.

Mechanism of Chiral Induction in Biliverdin Bis(amino Acid Methyl Esters).—The independence of the c.d. and electronic absorption spectra from concentration strongly favours an intramolecular induction mechanism. Thus, the chiral substituents positioned in the two side chains of compound (**3**)—(**8**) must influence the helicity of the bilatriene backbone across five bond lengths. Therefore a stretched conformation of the side chains in positions 8 and 12 is less likely. This would prevent close contact with the bilatriene moiety. Comparing the Δε values of the bis(alanine) derivative (**14**) with those of the monoalanine compound (**15**) show that the chiral induction of the two side chains is nearly additive for both c.d. bands in all solvents studied (Table 2). This implies a conformation in which the proximate side chains do not appreciably interact with each other and are situated above and below the bilatriene helix, respectively. Accordingly, the biliverdin-XIIIα derivative (**14**) adopts a conformation belonging to the C_2 point group.

The magnitude of the Δε values for a given bis(amino acid) derivative is largest in benzene and decreases in the order



Δε values (long wavelength band) of the bis(amino acid) derivatives (3)—(8) in various solvents (●, chloroform; ○, dichloromethane; ▲, ethanol; and △, tetrahydrofuran) *versus* Δε values of the same band measured in benzene

chloroform, dichloromethane, ethanol, and tetrahydrofuran (Table 2 and Figure) correlating with the dipole moment rather than the dielectric constant of the solvent. This sequence roughly parallels the increasing competition of intermolecular hydrogen bonds of the solvent. Clearly, the conformation postulated is stabilized by hydrogen bonds between the bilatriene moiety and its own side chains. In polar solvents these intramolecular associates are weakened and, simultaneously, a stretched arrangement of the side chains might become more important. The concomitant decrease of chiral induction is shown in the Figure where the Δε values of the long wavelength band of compounds (3)—(8) obtained in chloroform, dichloromethane, ethanol, and tetrahydrofuran solutions are plotted against the corresponding values in benzene.

One might argue that the solvent dependence of the c.d. observed is due to a solvent-dependent ratio of helical *versus* stretched bilatriene species. In fact, heterogeneity of biliverdin-IXα dimethyl ester (1) in solution has been detected by fluorescence techniques.[4,12,13] However, for equilibration of the two forms heating or sonicating[13] is necessary, indicating that the activation barrier is quite large. Therefore interconversion at ambient temperature must be slow on the n.m.r. time scale and it should be easily detectable if appreciable amounts (> 3%) of a second species were present. Nevertheless, the actual ^{1}H n.m.r. spectra of compounds (1), (3)—(11), (14), and (15) in chloroform solution do not give any hint at such heterogeneity in solution. In the case of the alanine derivative (14), which is best suited to scrutinize homogeneity because of its C_2-symmetry, additional recordings in [$^2H_8$]tetrahydrofuran and dideuteriodichloromethane led to the same result.

Upon formation of hydrogen bonds the bilatriene moiety can act both as hydrogen donor and acceptor. The same holds true for the residues in the side chains, the amide hydrogens serving as hydrogen donors and the ester carbonyl groups as acceptors. Obviously, both sites are involved in hydrogen bonding with the bilatriene skeleton. This follows from a comparison of the c.d. spectra of the pairs (4)/(10) and (3)/(9), respectively (Table 2). For both compounds (9) and (10) the Δε values are significantly decreased. Compound (10) differs from the phenylalanine derivative (4) by the absence of the methoxycarbonyl group which is replaced by a methyl group. Similarly, the lactic acid derivative (9) is devoid of the amide N–H group of the alanine derivative (3), carrying oxygen instead in this position. Compound (9) shows a very small c.d. in all solvents studied ($\Delta\varepsilon_{660}$ 0.3—1.3). Attenuation of the c.d. of the amphetamine derivative (10) is less pronounced indicating that the N–H amide association with the bilatriene backbone must be stronger and thus more important than that of the ester carbonyl group. Apparently, the presence of two association sites in the chiral substituents of the side chains is a prerequisite for an efficient influence on the bilatriene helicity. The amino acid residues bound to biliverdin thus serve as chiral 'clamps' on the bilatriene moiety.

Apart from the nature of the solvent the efficiency of chiral induction should also depend on the extent of distortion of the chiral intramolecular associate formed between the amino acid residues and the bilatriene backbone and thus should be influenced by the steric requirements of the amino acid. This is consistent with the enhancement of the Δε values of the leucine, tryptophan, and valine derivatives (6)—(8) in benzene as compared with the alanine, phenylalanine, and methionine derivatives (3)—(5) in the same solvent (Table 2, Figure). With the exception of compounds (5) and (7) the sequence observed for benzene holds true for the polar solvents. However, the deviations are not unexpected and most probably are due to additional interactions of the solvent with the polar substituents of the amino acid residues in the derivatives (5) and (7).

In conclusion our results clearly show that specific intramolecular interactions of biliverdin and bilatrienes in general are not restricted to high molecular proteins but do also occur in simple amino acid derivatives. Even in protic solvents like ethanol these interactions are largely preserved. However, only the population of conformers of opposite helicity is altered, and not the helical arrangement itself. Hence, the question arises if non-bonded chromophore–protein interactions are sufficient to force the chromophore in a stretched conformation, a geometry which has been suggested[1] for native biliproteins including phytochrome, phycocyanin, and phycoerythrin. To answer this question an investigation of oligopeptides and of the role of secondary structure is necessary. A study on that subject is in progress.

Experimental

M.p.s were taken with a Kofler–Reichert hot-stage apparatus. ^{1}H N.m.r. spectra (250 MHz; Fourier transform mode) were measured with a Bruker WM 250 instrument at 20 °C in spectroscopic grade deuteriochloroform unless stated otherwise. As internal standard tetramethylsilane was used. Mass spectra were taken with a Varian MAT 311A instrument equipped with spectrosystem 166 using the fast atom bombardment (f.a.b.) technique (butane-1,2,4-triol; Xe). Molecular masses in chloroform solution (*ca.* 10^{-2}M) were determined with a vapour pressure osmometer (Knauer). Electronic absorption spectra were measured at 20 °C with a Cary 15 spectrometer (0.1—2.0 cm quartz cuvettes). The c.d. spectra were taken with a Jobin Yvon Mark III instrument carrying thermostatted (20 ± 1 °C) cylindrical quartz cuvettes (0.05—2.0 cm). As solvents for electronic absorption and c.d. measurements spectroscopic grade (Uvasol; Merck) benzene, chloroform, dichloromethane, and ethanol were used. Dichloromethane and chloroform were chromatographed on aluminia prior to use. Tetrahydrofuran

Table 3. ^{1}H N.m.r. spectral data (δ) of compounds (**3**)—(**10**), (**14**), and (**15**) in deuteriochloroform of *ca.* 10^{-2}M solutions at 20 °C

Compound	Biliverdin moiety[a]	Acyl substituent R
(**3**)	6.81 (1 H, s), 6.62 (1 H, m), 6.52 (1 H, m), 6.15 (1 H, m), 6.05 (1 H, s), 5.99 (1 H, s), 5.67 (2 H, m), 5.45 (1 H, m), 2.94 (4 H, m), 2.43 (4 H, m), 2.19 (3 H, s), 2.11 (3 H, s), 2.09 (3 H, s), 1.89 (3 H, s)	6.72 (1 H, d, *J* 7.5 Hz), 6.64 (1 H, d, *J* 7.5 Hz), 4.51 (2 H, quintet, *J* 7.5 Hz), 3.67 (3 H, s), 3.66 (3 H, s), 1.33 (3 H, d, *J* 7.5 Hz), 1.32 (3 H, d, *J* 7.5 Hz)
(**4**)	6.79 (1 H, s), 6.66 (1 H, m), 6.51 (1 H, m), 6.15 (1 H, m), 6.03 (1 H, s), 5.98 (1 H, s), 5.66 (2 H, m), 5.45 (1 H, m), 3.00 (4 H, m), 2.39 (4 H, m), 2.16 (3 H, s), 2.08 (3 H, s), 2.05 (3 H, s), 1.88 (3 H, s)	7.19 (6 H, m), 7.02 (4 H, m), 6.69 (1 H, d, *J* 8.0 Hz), 6.61 (1 H, d, *J* 8.0 Hz), 4.77 (2 H, m), 3.65 (3 H, s), 3.63 (3 H, s), 2.88 (4 H, m)
(**5**)	6.70 (1 H, s), 6.57 (1 H, m), 6.45 (1 H, m), 6.09 (1 H, m), 5.98 (1 H, s), 5.90 (1 H, s), 5.62 (2 H, m), 5.39 (1 H, m), 2.89 (4 H, m), 2.40 (4 H, m), 2.09 (3 H, s), 2.04 (3 H, s), 2.02 (3 H, s), 1.82 (3 H, s)	6.86 (1 H, d, *J ca.* 7 Hz), 6.82 (1 H, d, *J ca.* 7 Hz), 4.57 (2 H, q, *J ca.* 7 Hz), 3.64 (6 H, s), 2.33 (4 H, m), *ca.* 2.0 (4 H, m), 1.97 (6 H, s)
(**6**)	6.65 (1 H, s), 6.56 (1 H, m), 6.42 (1 H, m), 6.10 (1 H, m), 5.98 (1 H, s), 5.92 (1 H, s), 5.59 (2 H, m), 5.38 (1 H, m), 2.87 (4 H, m), *ca.* 2.4 (4 H, m), 2.12 (3 H, s), 2.04 (3 H, s), 2.02 (3 H, s), 1.84 (3 H, s)	6.61 (1 H, d, *J* 7.5 Hz), 6.50 (1 H, d, *J* 7.5 Hz), 4.45 (2 H, m), 3.56 (3 H, s), 3.54 (3 H, s), *ca.* 2.4 (2 H, m), *ca.* 2.0 (4 H, m), 0.81 (12 H, d, *J ca.* 7 Hz)
(**7**)	6.66 (1 H, s), *ca.* 6.6 (1 H, m), 6.48 (1 H, m), 6.14 (1 H, m), 5.95 (1 H, s), 5.90 (1 H, s), 5.67 (2 H, m), 5.46 (1 H, m), 2.87 (2 H, m), 2.72 (2 H, m), 2.45 (2 H, m), 2.18 (2 H, m), 2.18 (3 H, s), 1.93 (3 H, s), 1.91 (6 H, s)	9.16 (2 H, s), 7.48 (2 H, m), 7.30 (2 H, m), 7.10 (4 H, m), 6.74 (2 H, s), *ca.* 6.6 (2 H, m), 4.58 (2 H, q, *J* 6.5 Hz), 3.61 (6 H, s), 3.19 (4 H, m)
(**8**)	6.70 (1 H, s), 6.63 (1 H, m), 6.45 (1 H, m), 6.14 (1 H, m), 5.98 (1 H, s), 5.93 (1 H, s), 5.51 (2 H, m), 5.39 (1 H, m), 2.90 (4 H, m), 2.48 (2 H, m), 2.30 (2 H, m), 2.13 (3 H, s), 2.06 (3 H, s), 2.02 (3 H, s), 1.85 (3 H, s)	6.64 (1 H, d, *J* 8.0 Hz), 6.49 (1 H, d, *J* 8.0 Hz), 4.37 (2 H, m), 3.58 (3 H, s), 3.57 (3 H, s), *ca.* 2.0 (2 H, m), 0.83 (3 H, d, *J* 7.5 Hz), 0.81 (3 H, d, *J* 7.5 Hz), 0.78 (3 H, d, *J* 7.5 Hz), 0.77 (3 H, d, *J* 7.5 Hz)
(**9**)	6.83 (1 H, s), 6.64 (1 H, m), 6.52 (1 H, m), 6.15 (1 H, m), 6.10 (1 H, s), 6.05 (1 H, s), 5.67 (2 H, m), 5.45 (1 H, m), 2.97 (4 H, m), 2.65 (4 H, m), 2.21 (3 H, s), 2.13 (3 H, s), 2.11 (3 H, s), 1.92 (3 H, s)	5.08 (2 H, q, *J* 7.5 Hz), 4.21 (4 H, q, *J* 7.0 Hz), 1.48 (6 H, d, *J* 7.5 Hz), 1.29 (6 H, t, *J* 7.0 Hz)
(**10**)	6.87 (1 H, s), 6.60 (1 H, m), 6.48 (1 H, m), 6.13 (1 H, m), 6.00 (1 H, s), 5.95 (1 H, s), 5.65 (2 H, m), 5.36 (1 H, m), 2.87 (4 H, m), 2.35 (4 H, m), 2.16 (3 H, s), 2.09 (3 H, s), 2.06 (3 H, s), 1.87 (3 H, s)	7.20 (6 H, m), 7.09 (4 H, m), 6.48 (1 H, d, *J* 7.5 Hz), 6.44 (1 H, d, *J* 7.5 Hz), 4.17 (2 H, quintet, *J ca.* 7 Hz), 2.69 (4 H, m), 1.03 (6 H, d, *J ca.* 7 Hz)
(**14**)[b]	6.76 (1 H, s), 6.60 (2 H, m), 5.98 (2 H, s), 5.67 (4 H, m), 2.93 (4 H, m), 2.43 (4 H, m), 2.06 (6 H, s), 1.93 (6 H, s)	6.74 (2 H, d, *J* 7.0 Hz), 4.51 (2 H, quintet, *J* 7.0 Hz), 3.70 (6 H, s), 1.33 (6 H, d, *J* 7.0 Hz)
(**15**)	6.77 (1 H, s), 6.61 (2 H, m), 6.02 (1 H, s), 6.01 (1 H, s), 5.68 (4 H, m), 2.94 (4 H, m), 2.59 (2 H, m), 2.43 (2 H, m), 2.09 (6 H, s), 1.93 (6 H, s)	6.35 (1 H, d, *J* 7.5 Hz), 4.57 (1 H, quintet, *J* 7.5 Hz), 3.71 (3 H, s), 3.67 (3 H, s), 1.34 (3 H, d, *J* 7.5 Hz)

[a] This part of the spectra is very similar to that of the corresponding dimethyl esters (**1**) and (**11**),[3,11] respectively. Therefore, most of the assignments of the resonance absorptions of compounds (**3**)—(**10**), (**14**), and (**15**) can simply be established by drawing a comparison. [b] Except for slight differences in chemical shifts, the spectra obtained in [$^2H_8$]tetrahydrofuran and CD_2Cl_2 are very similar.

(p.a.; Merck) was distilled twice from lithium aluminium hydride. Optical rotations used for checking the optical purity of the chiral starting materials were obtained with a Perkin-Elmer 241 instrument.

All reactions were carried out under argon. Column chromatography was performed on Kieselgel 60 (230—400 mesh; Merck) with chloroform (LiChrosolv; Merck) and ethyl acetate (p.a.; Merck) as eluants.

Biliverdin-IXα (**2**) was prepared from the isomer-free ester (**1**)[3,11] by saponification with sodium hydroxide in a mixture of water, tetrahydrofuran, and methanol in analogy to the synthesis of the XIIIα-isomer (**13**) using four equivalents of sodium hydroxide (see below).

Biliverdin-IXα Bis[(S)-*amino Acid Methyl Esters*] (**3**)—(**8**).—*General procedure.* To biliverdin-IXα (**2**) (58 mg, 0.1 mmol) dissolved in tetrahydrofuran (2 ml) were added successively under stirring the optically pure (*S*)-amino acid methyl ester hydrochloride[14] (0.3 mmol), *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodi-imide hydrochloride (Sigma) (60 mg, 0.31 mmol), and pyridine (25 μl, 0.3 mmol). After 24 h at room temperature chloroform (15 ml) and 0.01M aqueous acetic acid (10 ml) were added. The organic layer was washed twice with 0.01M aqueous acetic acid and subsequently with water. The chloroform extract was dried (Na_2SO_4) and the solvent evaporated off *in vacuo*. After column chromatography on silica gel [eluant ethyl acetate–chloroform (3:1 v/v)] the pure compounds were obtained. Yields for compounds (**3**)—(**8**) were 50—60%. Physical constants and spectral data are in Tables 1—4.

Preparation of Compounds (**9**) *and* (**10**).—For the synthesis and isolation of compounds (**9**) and (**10**) the general procedure described for biliverdin-IXα bis[(*S*)-amino acid methyl esters] was used starting from biliverdin-IXα (**2**) and (*S*)-(−)-ethyl lactate (Fluka; distilled) and (*R*)-(−)-amphetamine sulphate,[15] respectively. Purification of compound (**9**) on silica gel was performed with chloroform–ethyl acetate (9:1 v/v) as eluant. The yield of compound (**9**) was 25% and of compound (**10**) 60%. For physical constants and spectral data see Tables 1—4.

Biliverdin-XIIIα Mono- and Bis-[(S)-*alanine Methyl Ester*] (**14**) *and* (**15**).—A solution of the dimethyl ester (**11**)[3,11] (132 mg, 0.22 mmol) in tetrahydrofuran (5 ml), methanol (2 ml), and 1M aqueous sodium hydroxide (0.22 ml) was allowed to stand at room temperature for 4 h. The mixture was then poured into water (50 ml) and the pH adjusted to pH 5 with acetic acid. After centrifugation the gel obtained was dried. The amorphous powder (90 mg) was coupled with (*S*)-(+)-alanine methyl ester hydrochloride[14] (60 mg, 0.43 mmol) by means of the carbodi-

Table 4. Analytical data for compounds (3)—(10), (14), and (15)

Compound[a] (Formula)	M.p. (°C)[b]	Found (%) (Required) C	H	N	Molecular mass[c] $[M + 1]^+$
(3)	209—211	65.2	6.5	11.0	753
($C_{41}H_{48}N_6O_8$)		(65.4)	(6.4)	(11.2)	
(4)	165—168	70.4	6.1	9.4	905
($C_{53}H_{56}N_6O_8$)		(70.3)	(6.2)	(9.3)	
(5)	157	61.4	6.5	9.4[d]	873
($C_{45}H_{56}N_6O_8S_2$)		(61.9)	(6.5)	(9.6)	
(6)	138	67.6	7.1	10.2	837
($C_{47}H_{60}N_6O_8$)		(67.4)	(7.2)	(10.0)	
(7)	149—153	69.6	6.0	11.2	983
($C_{57}H_{58}N_8O_8$)		(69.6)	(5.9)	(11.4)	
(8)	191—193	66.5	6.9	10.5	809
($C_{45}H_{56}N_6O_8$)		(66.8)	(7.0)	(10.4)	
(9)	Oil	65.8	6.5	7.2	783
($C_{43}H_{50}N_4O_{10}$)		(66.0)	(6.4)	(7.2)	
(10)	185	75.2	6.7	10.1	817
($C_{51}H_{56}N_6O_4$)		(75.0)	(6.9)	(10.3)	
(14)	234—237	65.1	6.3	11.1	753
($C_{41}H_{48}N_6O_8$)		(65.4)	(6.4)	(11.2)	
(15)	186—188	66.7	6.4	10.5	682
($C_{38}H_{43}N_5O_7$)		(66.9)	(6.4)	(10.3)	

[a] Due to the large absorbance at λ 589 nm no optical rotation was measured. [b] From benzene–ethyl acetate. [c] By f.a.b. mass spectrometry. [d] S, 7.7% (7.3%).

imide method as described above. After chromatography on silica gel (see general procedure) starting material (11) (30 mg) and compounds (14) (15 mg, 12%) and (15) (11 mg, 10%) were obtained. For physical constants and spectral data see Tables 1—4.

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References

1 For reviews see H. Scheer, *Angew. Chem.*, 1981, **93**, 230; *Angew. Chem., Int. Ed. Engl.*, 1981, **20**, 241; T. W. Goodwin, 'Chemistry and Biochemistry of Plant Pigments,' Academic Press, New York, 1976, 2nd edn., vol. 1, p. 328.
2 G. Blauer and B. Zvilichovsky, *Isr. J. Chem.*, 1973, **11**, 435; G. Blauer and G. Wagnière, *J. Am. Chem. Soc.*, 1975, **97**, 1949; *Arch. Biochem. Biophys.*, 1975, **170**, 375; A. A. Lamola, S. E. Braslavsky, K. Schaffner, and D. A. Lightner, *Photochem. Photobiol.*, 1983, **37**, 263.
3 H. Lehner, S. E. Braslavsky, and K. Schaffner, *Justus Liebigs Ann. Chem.*, 1978, 1990.
4 S. E. Braslavsky, A. R. Holzwarth, E. Langer, H. Lehner, J. I. Matthews, and K. Schaffner, *Isr. J. Chem.*, 1980, **20**, 196.
5 A. R. Holzwarth, E. Langer, H. Lehner, and K. Schaffner, *J. Mol. Struct.*, 1980, **60**, 367; *Photochem. Photobiol.*, 1980, **32**, 17.
6 H. Lehner, C. Krauss, and H. Scheer, *Z. Naturforsch., Teil B*, 1981, **36**, 735.
7 G. Blauer, *Isr. J. Chem.*, 1983, **23**, 201.
8 E. Langer, H. Lehner, W. Rüdiger, and B. Zickendraht-Wendelstadt, *Z. Naturforsch., Teil C*, 1980, **35**, 367; H. Lehner and H. Scheer, 1983, **38**, 353; T. Brandlmeier, H. Lehner, and W. Rüdiger, *Photochem. Photobiol.*, 1981, **34**, 69.
9 J. C. Lagarias, A. N. Glazer, and H. Rapoport, *J. Am. Chem. Soc.*, 1979, **101**, 5030; J. C. Lagarias and H. Rapoport, *ibid.*, 1980, **102**, 4821; R. W. Schoenleber, S.-L. Leung, D. J. Lundell, A. N. Glazer, and H. Rapoport, *ibid.*, 1983, **105**, 4072.
10 H. Lehner, W. Riemer, and K. Schaffner, *Justus Liebigs Ann. Chem.*, 1979, 1798.
11 R. Bonnett and A. F. McDonagh, *Chem. Commun.*, 1970, 237, 238.
12 A. R. Holzwarth, H. Lehner, S. E. Braslavsky, and K. Schaffner, *Justus Liebigs Ann. Chem.*, 1978, 2002.
13 A. R. Holzwarth, J. Wendler, K. Schaffner, V. Sundström, A. Sandström, and T. Gillbro, *Isr. J. Chem.*, 1983, **23**, 223.
14 K.-H. Deimer in Houben Weyl, Vol. 15/1, 4th edn., ed. E. Wünsch, Thieme, Stuttgart, 1974, p. 315.
15 A. W. Schrecker, *J. Org. Chem.*, 1957, **22**, 33; F. M. Jaeger and J. A. van Dijk, *Proc. Acad. Sci. Amsterdam*, 1941, **44**, 26.

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