Complete Chiral Discrimination in Bilipeptides: the Chiroptical Properties of the Bilatriene Helix

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Biliverdin oligopeptides have been prepared with the aim of complete chiral discrimination of the conformationally labile bilatriene helix. Evidence is given that appropriately constituted biliverdin-tripeptides and -tetrapeptides exhibit diastereoisomeric homogeneity in solution. To ascertain the homochirality of the bilatriene helices present in a bilipeptide two independent approaches were used: invariability of the chiroptical properties towards (i) successive peptide chain lengthening and towards (ii) the polarity of the solvent. The c.d. parameters of the inherently chiral bilatriene (*P*)-helix are thus evaluated to be $\Delta\varepsilon$ +100 to +110 and $\Delta\varepsilon$ −130 to −150 for the red and blue band, respectively. These parameters belong to a bilatriene helix whose torsional angles closely resemble those of biliverdin dimethyl ester.

Biliverdin, like many other bilatrienes in the *Z,Z,Z*-configuration, preferentially adopts a helical conformation in solutions.[1-3] Owing to the low interconversion barrier[4] resolution of the inherently chiral helices is not possible by simple means. In the course of our systematic investigations into the conformational behaviour of bilipeptides[5-8] we have found that chiral discrimination[9] of the bilatriene helices monitored by c.d. spectroscopy in the absorption region of the chromophore, may be mediated by covalently bound chiral amino acid derivatives or peptides. This kind of resolution occurs intramolecularly by non-bonding interaction of the kinetically labile bilatriene backbone with its own side-chains. As has been shown recently in more detail[7,8] the extent of discrimination and the nature of the constituents of the peptide moiety are strongly interdependent. In addition, the helical excess† is particularly sensitive towards the polarity of the solvent considered. Exceptions are found with peptides and amino acid derivatives whose discriminatory efficiencies are poor *a priori* due to special groups present which prevent effective interactions with the bilatriene moiety. However, absolute values for the extent of discrimination in bilipeptides could not be given until now since the chiroptical properties of the homochiral bilatriene helices are still unknown. This prompted us to elucidate the appropriate conditions and the amino acid constituents necessary for complete chiral discrimination.

Results and Discussion

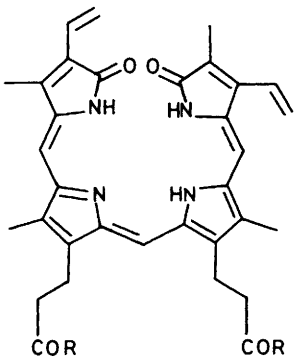
From our detailed study on chiral, optically active bilipeptides and amino acid derivatives[5-8] it is evident that successive chain lengthening of the peptide moieties may increase the enantiomeric excess of the bilatriene helices of one distinct chirality. Thus, an invariance of the chiroptical properties of two homopeptides differing only in their number of (*S*)-amino acid constituents should be indicative of their homochirality. For this purpose the alanine building unit turns out to be most suitable. It was preferred over valine because of its larger discriminatory efficiency if part of a peptide chain.

The c.d. parameters and molar rotations of the bilipeptides (**1**)—(**3**) obtained for solutions in chloroform–methanol (98:2 v/v) are compiled in Table 1. These compounds differ solely in their number of alanine entities. If compared with the dipeptide (**1**) the values obtained for the tripeptide (**2**) are dramatically enhanced. However, the parameters of the tetrapeptide (**3**) exhibit no further substantial changes although on account of

Table 1. C.d. [$\Delta\varepsilon_{max.}$/l mol^{-1} cm^{-1} ($\lambda_{max.}$/nm)],[a] molar rotations $[M]^{20}_{546}$ (deg mol^{-1}),[b] and u.v.–visible absorption spectra [$\varepsilon_{max.}$/l mol^{-1} cm^{-1} ($\lambda_{max.}$/nm)][a] of biliverdin peptides (**1**)—(**3**) in chloroform–methanol (98:2 v/v) of *ca.* 3 × 10^{-5}M solutions at 20 °C[a,c]

Compound	C.d.	$[M]^{20}_{546}$	U.v.–visible
(**1**)	+21.4(650) −36.6(379)	−55 000	13 600(650) 48 300(379)
(**2**)	+99.5(655) −137.6(377)	−223 000	13 500(652) 46 000(377)
(**3**)	+100.0(650) −142.4(378)	−205 000	12 500(650) 43 700(377)

[a] $\lambda_{max.}$ ± 5 nm (red band), ±2 nm (blue band); all spectra were run at least in duplicate; reproducibility of ε and $\Delta\varepsilon$-values at least ±5%. [b] ±5 000° for (**1**) and (**2**), ±10 000° for (**3**). [c] Because of the poor solubility of (**3**) the values refer to 10^{-6}M solutions.



(**1**) R = (*S*)-Ala-(*S*)-Ala-OMe

(**2**) R = (*S*)-Ala-(*S*)-Ala-(*S*)-Ala-OMe

(**3**) R = (*S*)-Ala-(*S*)-Ala-(*S*)-Ala-(*S*)-Ala-OMe

(**4**) R = (*S*)-Leu-Gly-(*S*)-Pro-OMe

(**5**) R = (*S*)-Val-(*S*)-Pro-(*S*)-Ala-(*S*)-Val-OMe

(**6**) R = (*S*)-Ala-(*S*)-Pro-(*S*)-Ala-(*S*)-Val-OMe

† Here and elsewhere the terms helical excess, enantiomeric excess, and homochirality refer to the bilatriene moiety only, omitting the chirality elements present in the peptide chains.

Table 2. C.d. spectra [$\Delta\varepsilon_{max.}$/l mol^{-1} cm^{-1} ($\lambda_{max.}$/nm)][a] and molar rotations $[M]_{546}^{20}$ (deg mol^{-1})[b] of biliverdin peptides (**4**)—(**6**) in various solvents of *ca.* 3 × 10^{-5}M solutions at 20 °C

	Benzene		Chloroform		Chloroform–methanol (98:2 v/v)		Ethanol	
Compound	c.d.	$[M]_{546}^{20}$	c.d.	$[M]_{546}^{20}$	c.d.	$[M]_{546}^{20}$	c.d.	$[M]_{546}^{20}$
(**4**)	+102.9(662) −147.0(381)	−219 000	+88.9(661) −134.9(378)	−190 000	+80.3(660) −119.3(379)	−161 000	+47.0(666) −78.1(379)	−109 000
(**5**)	+101.6(660) −140.0(380)	−225 000	+108.8(661) −149.1(378)	−238 000	+101.2(660) −137.7(378)	−220 000	+52.8(662) −64.0(378)	−122 000
(**6**)	+101.3(655) −140.0(380)	−219 000	+110.0(658) −143.0(379)	−237 000	+100.5(660) −130.1(378)	−218 000	+51.6(660) −66.0(378)	−114 000

[a] As Table 1. [b] ±5 000°.

Table 3. U.v.–visible absorption spectra [$\varepsilon_{max.}$/l mol^{-1} cm^{-1} ($\lambda_{max.}$/nm)][a] of biliverdin peptides (**4**)—(**6**) in various solvents of *ca.* 3 × 10^{-5}M solutions at 20 °C

Compound	Benzene	Chloroform	Chloroform–methanol (98:2 v/v)	Ethanol
(**4**)	15 100(664) 47 300(381)	13 800(667) 46 800(380)	14 500(658) 46 400(380)	14 300(658) 47 100(379)
(**5**)	13 500(660) 45 000(380)	13 100(661) 46 700(379)	13 200(662) 44 600(380)	13 000(663) 45 900(378)
(**6**)	13 400(660) 44 300(380)	12 700(662) 45 100(379)	12 800(662) 45 300(380)	12 800(664) 46 700(379)

[a] See Table 1.

the larger number of co-ordination sites present discriminatory forces can be expected to be increased. These observations strongly suggest that chiral discrimination in the tripeptide (**2**) has already reached the highest value. Hence, if the bilatriene helices in the bilipeptides (**2**) and (**3**) are enantiomerically homogeneous their chiroptical properties in the absorption region of the chromophore essentially reflect those of the bilatriene helix itself. Unfortunately, owing to the generally poor solubility of biliverdin homopeptides void of additional polar groups only one solvent [chloroform–methanol (98:2 v/v)]* could be used. Thus, experiments with the aim of investigating the solvent dependence of the chiroptical properties of compounds (**2**) and (**3**) could not be performed. For this purpose and in order to corroborate the conclusions from successive lengthening of the peptide entities the heteropeptides (**4**)—(**6**) bearing the advantage of much better solubility in a variety of solvents were taken into consideration. The Cotton effects of the tripeptide (**4**) decrease with increasing polarity of the solvent (Table 2). This behaviour is reminiscent of biliverdin amino acids and peptides for which mutual interactions of the bilatriene moiety and its side-chains can be regarded as large.[7,8] Clearly the solvent dependent parameters of (**4**) reflect an attenuation of discriminatory forces due to intermolecular competition of the surrounding medium. Strikingly, the chiroptical properties of the heteropeptide (**4**) for benzene solution are very close to those obtained for the homopeptides (**2**) and (**3**). On the other hand, the tetrapeptide (**5**) for benzene solution likewise exhibits a c.d. spectrum and molar rotation similar to those of the quite differently constituted compounds (**2**)—(**4**). However, the typical solvent dependence of the chiroptical properties observed for the tripeptide (**4**) is partly lost. Within experimental fluctuation the parameters of (**5**) measured for benzene and chloroform–methanol solutions are identical; for chloroform solution even a slight enhancement is observed. Only in ethanol does a pronounced decrease of the Δε-values and the molar rotation by *ca.* 50% occur. Nevertheless, this indicates the presence of one helical form to *ca.* 75% even in this protic solvent. If the *N*-terminal amino acid valine in compound (**5**) is substituted by the less bulky alanine, again no substantial changes of the chiroptical properties take place as can be seen from comparison with compound (**6**), although decreasing size, especially that of the *N*-terminal amino acid, in general gives rise to an increase of discriminatory forces.[8] These results are further evidence in support of complete chiral discrimination of the bilatriene helices in compounds (**2**) and (**3**) and the heteropeptides (**5**) and (**6**) in the appropriate solvents. Therefore, the data for bilipeptides (**2**) and (**3**) (both for solutions in chloroform–methanol), (**4**) (benzene solution only), and (**5**) and (**6**) (all solvents except ethanol) displayed in Tables 1 and 2 essentially reflect the chiroptical properties of the bilatriene helix. The absolute values are thus Δε (*ca.* 660 nm) + 100 to +110, Δε (*ca.* 380 nm) −137 to −149, corresponding to rotational strengths *R* of *ca.* 4.0 × 10^{-38} and *ca.* −4.6 × 10^{-38} erg cm^3 for the red and blue band, respectively. The molar rotation amounts to $[M]_{546}^{20}$ −205 000 to −238 000°. Most likely, the slightly larger values for the chromopeptides (**5**) and (**6**) found for chloroform solutions simply reflect a solvent dependence of the chiroptical properties, a phenomenon which is common for c.d. spectra of very polar molecules and may occur even with rigid compounds.[10]

From theoretical studies[11] it has been concluded that in general a bilatriene showing a positive and a negative sign in the visible and near-u.v. c.d. bands, respectively, is associated with (*P*)-helicity. Accordingly, the chiroptical properties derived for the bilatriene moiety belong to a right-handed helix. Even if the helical excess in bilipeptides varies with the nature of the chiral substituents bound to the propionic side-chains, for all bilipeptides and amino acid derivatives with (*S*)-amino acids investigated so far the phenotype of the c.d. spectrum and thus the predominence of the (*P*)-helical form is retained.

Although the u.v.–visible absorption spectra of bilatrienes, expecially the ratio of absorptivities of the two electronic absorption bands, are particularly sensitive to conformational

* In pure chloroform or methanol compounds (**2**) and (**3**) are insoluble.

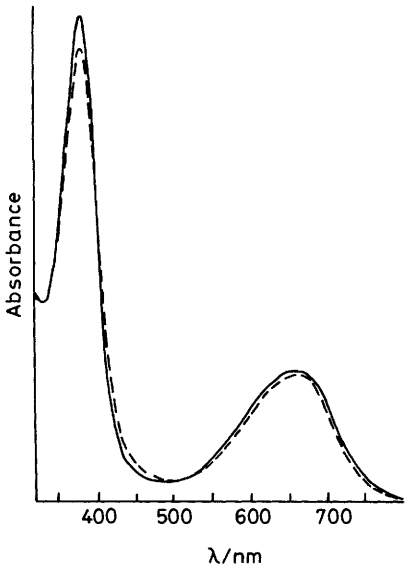


Figure. U.v.–visible absorption spectra of 3.0×10^{-5}M solutions of biliverdin-IXα dimethyl ester (——) and (**5**) (– – – –) in chloroform at 20 °C

changes[11] the chromopeptides considered here exhibit very similar parameters with respect to both band position and absorptivity (Tables 1 and 3). Moreover, they closely resemble those of other bilipeptides, biliverdin amino acid esters, or even biliverdin dimethyl ester.[4,5,7,8] To give an illustrative example of this similarity the u.v.–visible absorption spectra of the tetrapeptide (**5**) and biliverdin-IXα dimethyl ester are shown in the Figure. These findings not only suggest that the presence of stretched species can be excluded for solutions of peptides (**1**)—(**6**) but also indicate preservation of the geometry of the flexible bilatriene helix on substitution in the propionic side-chains. Accordingly, the values given above reflect the properties of a bilatriene helix whose torsional angles and pitch are close to those of other biliverdin derivatives, irrespective of substitution. On the other hand, a quite different geometry of the bilatriene helix might be the reason for the partly poor agreement of our c.d. parameters with those reported for an N-21—N-24 methano-bridged biliverdin. While the long-wavelength band[12] [Δε (700 nm) $+100 \pm 20$] is in accord with our value the c.d. in the near u.v. region[12] [Δε (380 nm) -30 ± 6] markedly deviates. However, this discrepancy becomes less surprising in view of the u.v.–visible spectrum reported for this bridged compound [ε (695 nm) 3 700, ε (360 nm) 21 000][12] being quite different from that of open-chain biliverdins described here and in previous studies.

Experimental

General Directions.—M.p.s were determined with a Kofler–Reichert hot-stage apparatus. ^{1}H N.m.r. spectra (250 MHz; Fourier transform mode) were recorded with a Bruker WM 250 instrument at 20 °C for solutions in [$^2H_6$]DMSO and CF_3CO_2D using $SiMe_4$ as internal reference. Fast atom bombardment mass spectrometry (f.a.b.m.s.) was performed with a Varian MAT 311A instrument equipped with spectrosystem 166 (butane-1,2,4-triol; Xe). U.v.–visible spectra were measured with a Perkin-Elmer Lambda 7 spectrometer (1—10 cm quartz cuvettes). The c.d. spectra were taken with a Jobin Yvon Mark III instrument carrying cylindrical quartz cuvettes (0.5—10 cm). Optical rotations (10 cm path length) were obtained with a Perkin-Elmer 241 instrument. All spectroscopic measurements were carried out in thermostatted cell compartments (20 ± 1 °C). As solvents for optical rotations, u.v.–visible, and c.d. measurements spectroscopic grade benzene, methanol, ethanol (all Uvasol; Merck), and chloroform (LiChrosolv; Merck) were used. To prevent spectral interference by protonated species[6] due to traces of acids occasionally present in the solvents triethylamine (1 μl) was added to solutions prior to spectroscopic measurement. This addition does not cause any spectral changes if acid impurities are absent.

Starting Materials.—The synthesis of isomer-free biliverdin-IXα has been described elsewhere.[2] The oligopeptides used for syntheses have been prepared *via* the phosphorazo[13] and/or the hydroxysuccinimide[14] method. Their enantiomeric purity (94—98%) was established by both ^{1}H n.m.r. and g.l.c.[15]

Synthesis of Bilipeptides.—The bilipeptides (**1**)—(**6**) were prepared according to the general procedure given in ref. 8, starting from biliverdin-IXα,[2] the appropriate peptide ester hydrochloride, and *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodi-imide hydrochloride (Merck). After column chromatography [Kieselgel 60 (230—400 mesh), Merck] compounds (**1**)—(**6**) were obtained (yield 40—60%). As eluants chloroform–methanol (97 : 3 v/v) [(**1**), (**2**), (**4**)—(**6**)] and chloroform–methanol–trifluoroacetic acid (90 : 9 : 1 v/v) [(**3**)] were used. For characterisation of compounds (**1**) and (**2**) and further experimental details see ref. 8.

Biliverdin-IXα *bis*-[(S)-*alanyl*-(S)-*alanyl*-(S)-*alanyl*-(S)-*alanine methyl ester*] (**3**). This compound was prepared from biliverdin-IXα and (−)-(*S*)-alanyl-(*S*)-alanyl-(*S*)-alanyl-(*S*)-alanine methyl ester hydrochloride {$[\alpha]_D^{20}$ −81.1° (*c* 2 in MeOH)}; no m.p., gradually decomposing on heating to 300 °C; *m/z* 1 179 (M^+ + H); δ(CF_3CO_2D) biliverdin moiety: 7.69 (1 H, s), 6.68 (2 H, m), 6.59 (1 H, s), 6.48 (1 H, s), 6.41 (1 H, m), 5.90 (3 H, m), 3.34 (4 H, m), 2.86 (4 H, m), 2.35 (3 H, s), 2.32 (3 H, s), 2.30 (3 H, s), and 2.19 (3 H, s); acyl substituents: 4.76 (6 H, q, *J ca.* 7 Hz), 4.71 (2 H, q, *J ca.* 7 Hz), 3.96 (6 H, s), 1.55 (18 H, m), and 1.47 (6 H, d, *J* 6.8 Hz); for c.d., u.v.–visible spectrum, and molar rotation see Table 1.

Biliverdin-IXα *bis*-[(S)-*leucinylglycinyl*-(S)-*proline methyl ester*] (**4**). This compound was prepared from biliverdin-IXα and (−)-(*S*)-leucinylglycinyl-(*S*)-proline methyl ester hydrochloride {$[\alpha]_D^{20}$ −52.4° (*c* 1.3 in MeOH)}; m.p. 180—185 °C; *m/z* 1 145 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.88 (1 H, s), 6.83 (1 H, m), 6.57 (1 H, m), 6.17 (1 H, s), 6.16 (1 H, s), 6.10 (1 H, m), 5.71 (2 H, m), 5.40 (1 H, m), 2.82 (4 H, m), *ca.* 2.5 (2 H, m), 2.37 (2 H, m), 2.18 (3 H, s), 2.09 (3 H, s), 2.06 (3 H, s), and 1.83 (3 H, s); acyl substituents: 7.96 (4 H, m), 4.30 (4 H, m), 3.95 (2 H, m), 3.71 (2 H, m), 3.60 (6 H, s), *ca.* 3.4 (4 H, m), *ca.* 2.1 (2 H, m), *ca.* 1.9 (4 H, m), *ca.* 1.4 (8 H, m), 0.79 (6 H, d, *J* 6.5 Hz), and 0.75 (6 H, d, *J* 6.5 Hz); for c.d., u.v.–visible spectrum, and molar rotation see Tables 2 and 3.

Biliverdin-IXα *bis*-[(S)-*valyl*-(S)-*prolyl*-(S)-*alanyl*-(S)-*valine methyl ester*] (**5**). This compound was prepared from biliverdin-IXα and (−)-(*S*)-valyl-(*S*)-prolyl-(*S*)-alanyl-(*S*)-valine methyl ester hydrochloride {$[\alpha]_D^{20}$ −92.7° (*c* 2 in MeOH)}; m.p. 150—160 °C; *m/z* 1 343 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.91 (1 H, s), 6.83 (1 H, m), 6.56 (1 H, m), 6.14 (1 H, s), 6.11 (1 H, s), 6.09 (1 H, m), 5.69 (2 H, m), 5.38 (1 H, m), 2.80 (4 H, m), 2.37 (4 H, m), 2.17 (3 H, s), 2.07 (3 H, s), 2.05 (3 H, s), and 1.82 (3 H, s); acyl substituents: 8.05 (2 H, d, *J* 8.0 Hz), 8.00 (2 H, d, *J* 7.9 Hz), 7.96 (2 H, d, *J* 7.9 Hz), 4.34 (2 H, d, *J* 7.9 Hz), *ca.* 4.3 (2 H, m), 4.29 (2 H, quintet, *J* 7.9 Hz), 4.15 (2 H, dd, J_1 7.9, J_2 6.9 Hz), 3.69 (2 H, m), 3.63 (6 H, s), *ca.* 3.5 (2 H, m), *ca.* 2.0 (4 H, m), *ca.* 1.9 (8 H, m), 1.19 (6 H, d, *J* 7.8 Hz), 0.86 (6 H, d, *J* 6.5 Hz), 0.85_5 (6 H, d, *J* 6.5 Hz), 0.85 (6 H, d, *J* 6.5 Hz), and 0.79 (6 H, d, *J* 6.5 Hz); for c.d., u.v.–visible spectrum, and molar rotation see Tables 2 and 3.

Biliverdin-IXα *bis*-[(S)-*alanyl*-(S)-*prolyl*-(S)-*alanyl*-(S)-*valine methyl ester*] (**6**). This compound was prepared from biliverdin-IXα and (−)-(*S*)-alanyl-(*S*)-prolyl-(*S*)-alanyl-(*S*)-valine methyl ester hydrochloride {$[\alpha]_D^{20}$ −110.4° (*c* 1.3 in MeOH)}; m.p. 152—160 °C; *m/z* 1 287 (M^+ + H); δ ([$^2H_6$]DMSO) biliverdin moiety: 6.91 (1 H, s), 6.82 (1 H, m), 6.56 (1 H, m), 6.13 (1 H, s), 6.10 (1 H, s), 6.07 (1 H, m), 5.69 (2 H, m), 5.40 (1 H, m), 2.80 (4 H, m), 2.33 (4 H, m), 2.16 (3 H, s), 2.08 (3 H, s), 2.06 (3 H, s), and 1.82 (3 H, s); acyl substituents: 8.12 (2 H, d, *J* 7.6 Hz), 7.95 (2 H, d, *J* 7.5 Hz), 7.93 (2 H, d, *J* 7.5 Hz), 4.49 (2 H, quintet, *J* 7.2 Hz), 4.30 (4 H, m), 4.14 (2 H, t, *J* 7.4 Hz), 3.62 (6 H, s), *ca.* 3.5 (4 H, m), *ca.* 2.0 (2 H, m), *ca.* 1.9 (8 H, m), 1.20 (6 H, d, *J* 6.9 Hz), 1.14 (6 H, d, *J* 6.9 Hz), 0.89 (6 H, d, *J* 7.1 Hz), and 0.88 (6 H, d, *J* 7.1 Hz); for c.d., u.v.–visible spectrum, and molar rotation see Tables 2 and 3.

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