Peptide-mediated Conformational Changes in Bilipeptides: Evidence for the Occurrence of Stretched Species

Daniel Krois and Harald Lehner*
Institut für Organische Chemie der Universität Wien, Währingerstraße 38, A-1090 Wien, Österreich

Flexible biliverdins covalently bound to tripeptides of the sequence Pro-X-Y exhibit a remarkable tendency to adopt stretched conformations. This contrasts with the behaviour of the great many bilipeptides synthesized so far, preserving the (*Z,Z,Z,syn,syn,syn*) geometry. The conformational changes induced are strongly solvent dependent and sensitive to the steric requirements of the amino acid consituent X. In biliverdin bis(tripeptides) carrying two peptide entities in close proximity, the latter influence is superimposed by intramolecular peptide–peptide interchain interactions. In these cases chromophore stretching occurs only if the amino acid entity X becomes more bulky. Conformational changes proceed through rotation around the C(5) methine single bond, thus forming (*Z,Z,Z,anti,syn,syn*) conformers. The outstanding behaviour of Pro-X-Y peptide esters in favouring stretched conformers in poorly hydrogen-bonding solvents is ascribed to the formation of γ-bends. The conclusions drawn rest upon a combinatory evaluation of UV–VIS, CD, and ^{1}H NMR spectra of 18 biliverdin peptides, including conformationally mobile and rigid bilatriene chromophores.

Biliproteins serve as light-harvesting pigments in certain bacteria and algae and are also involved in photomorphogenesis of higher plants. They contain a bile pigment chromophore whose conformation changes if the influence of the covalently bound protein is removed by denaturation or chemical cleavage. This is reflected in the pronounced differences in photophysical properties such as UV–VIS spectra. To simulate these phenomena various means have been used.[1–5] However, the biologically most relevant approach to changing the preferred coiled conformation, by covalent binding to peptides, has been unsuccessful until now. Thus the peptide entities, out of the great many biliverdin oligopeptides synthesized so far, have been shown to preserve and even to stabilise the (*syn, syn, syn*) conformation of the flexible (*Z,Z,Z*) tetrapyrrolic chromophore.[1,6–10] The influence of the peptide is thus restricted to an inversion of all torsional angles of the *M* helical arrangement, equivalent to the formation of an excess of *P* helical species. Thereby discriminatory forces may become quite large so that the helical excess approaches 100% in some cases. In consequence it has been suspected that forcing of the chromophore into a stretched conformation would require a distinct secondary structure of the peptides in solution.[8] In continuing our systematic conformational investigations, we recently synthesized a set of biliverdin tripeptide esters possessing the sequence Pro-X-Y which prefer to adopt a stretched conformation. Some of our results will be given in this report.

Results and Discussion

UV–VIS and CD Spectra of Biliverdin Bis(tripeptides).—The UV–VIS spectra (in benzene) of the bis(tripeptides) (**2**) (R = Pro-Ala-Ala-OMe) and (**3**) (R = Pro-Ala-Val-OMe) derived from biliverdin-IXα (BV-IXα) (Table 1) still compare with those of other bilipeptides reported so far.[6–9] The relative intensities of their two main bands centred around λ 380 nm and λ 660 nm, respectively, are indicative of a helical (*Z,Z,Z,syn,syn,syn*) geometry of the conformationally labile bilatriene backbone. This is expressed by the ratios of dipole strengths $f = D_{UV}/D_{VIS}$ which are close to that of the reference (**1**) (*f* 2.41). Both parameters, band positions and intensity ratios *f*, do not substantially change if the hydrogen-bonding ability of the solvent increases. The magnitude of the pertinent visible CD bands and molar rotations in benzene (Table 2) is characteristic of complete chiral discrimination between *M* and *P* helical bilatriene antipodes.[9]

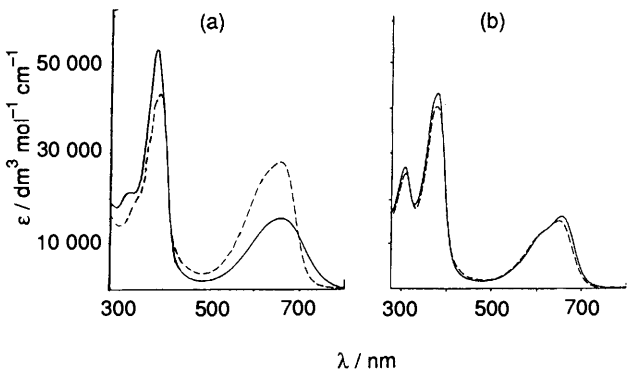
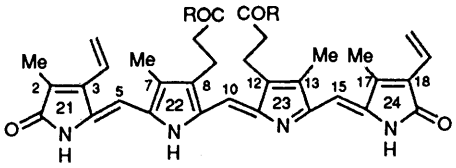


Figure 1. UV–VIS spectra of (a) compounds (**1**) (——) and (**8**) (– – – –) and (b) compounds (**10**) (——) and (**11a**) (– – – –) in benzene for *ca.* 3×10^{-5} mol dm^{-3} solutions at 20 °C.

In contradistinction, the UV–VIS spectra of the biliverdin-IXα bis(tripeptides) (**4**)–(**8**) in benzene differ in two main respects from those of compounds (**2**) and (**3**) (Table 1). One distinguishing feature is their pronounced decrease in relative intensity *f* of the two main absorption bands; *f* (1.3–1.7). Apparently, decrease in *f*-values is associated with the steric requirements of the amino acid entity X following proline, while for the *C*-terminal residue Y not even configuration has a noticeable effect on *f*. While the intensity of the visible band of compounds (**2**) and (**3**) (R = Pro-Ala-Y) remains essentially unchanged, if compared with that of (**1**) (R = OMe) it exhibits considerable enhancement in compounds (**4**), (**5**), and (**6**) (R = Pro-Leu-Y) and becomes twice as large in compounds (**7**) and (**8**) (R = Pro-Val-Y) [Table 1 and Figure 1(a)]. Simultaneously the pertinent UV absorption bands show a general lowering of dipole strength. The spectral changes observed for benzene solutions are far less pronounced for chloroform solutions and become very small if ethanol is the solvent. This striking solvatochromism comprises a further outstanding property of compounds (**4**)–(**8**).

Table 1. UV–VIS spectra [ε_{max}/dm^3 mol^{-1} cm^{-1} (λ_{max}/nm)] of compound (**1**) and BV-IXα-bis(peptides) (**2**)–(**9**) in various solvents.[a] The *f*-factor refers to the corresponding quotient of dipole strengths D_{UV}/D_{VIS}.[b,c]



	Benzene		Chloroform		Ethanol	
	ε (λ)	*f*	ε (λ)	*f*	ε (λ)	*f*
(**1**) R = OMe	16 400 (660) 55 400 (381)	2.41	14 800 (661) 55 200 (379)	2.62	15 200 (665) 56 200 (376)	2.62
(**2**) R = Pro-Ala-Ala-OMe	14 200 (656) 40 000 (382)	2.42	15 800 (651) 47 000 (382)	2.32	14 200 (658) 51 100 (378)	2.58
(**3**) R = Pro-Ala-Val-OMe	14 700 (654) 39 600 (383)	2.37	16 100 (651) 45 900 (380)	2.26	14 200 (656) 48 800 (378)	2.58
(**4**) R = Pro-Leu-Gly-OEt	21 200 (645) 43 900 (383)	1.68	16 900 (652) 48 900 (380)	2.10	14 000 (654) 51 500 (379)	2.57
(**5**) R = Pro-Leu-Leu-OMe	22 200 (643) 43 200 (383)	1.70	22 100 (647) 49 500 (382)	1.76	16 500 (651) 52 300 (378)	2.30
(**6**) R = Pro-Leu-Val-OMe	22 400 (642) 40 100 (384)	1.60	20 400 (650) 47 300 (383)	1.81	14 800 (654) 45 900 (379)	2.38
(**7**) R = Pro-Val-Val-OMe	27 500 (655) 43 700 (387)	1.47	23 100 (652) 47 900 (383)	1.72	16 000 (651) 51 000 (378)	2.33
(**8**) R = Pro-Val-(*R*)-Val-OMe	27 900 (658) 42 400 (388)	1.37	22 600 (652) 48 000 (383)	1.69	15 800 (649) 50 100 (378)	2.26
(**9**) R = Leu-Pro-Leu-Leu-OMe	13 600 (662) 46 900 (380)	2.75	13 200 (666) 49 000 (378)	2.76	13 300 (666) 46 500 (379)	2.78

[a] *ca.* 3 × 10^{-5} mol dm^{-3} solutions, 20 °C; spectral data did not change within the range 10^{-3}–10^{-6} mol dm^{-3} solutions. Configuration of α-amino acids refers to (*S*)-chirality if not stated otherwise. [b] For determination of dipole strengths of the visible and first UV band, integration was performed from λ 800 nm to λ 500 nm, and from λ 480 nm to λ 330 nm (cut-off), respectively. [c] Note that a rapid equilibrium exists between the N(22)-H tautomer (formula) and the N(23)-H tautomer (not shown).

Despite these considerable changes in intensity in benzene and chloroform, the band positions are less affected. On considering compounds (**1**)–(**8**) in order, the UV-band is slightly shifted bathochromically if *f* decreases (maximum deviation 7 nm). No correlation of *f* with λ_{max}-values is found for the visible band. Even if a hypsochromic shift of *ca.* 15–18 nm occurs when X is leucyl as in compounds (**4**)–(**6**), differences are smallest (2–5 nm) for compounds (**7**) and (**8**) exhibiting the smallest *f*-values.* On the other hand, solvent dependence of band positions does not exceed that observed for other bilatrienes. Owing to the sensitivity of UV–VIS spectra of bilatrienes towards conformational changes[1] the outstanding behaviour of compounds (**4**)–(**8**) in these aprotic solvents reflects a peptide-mediated torsion around one or more of the C(5), C(10), or C(15) methine single bonds. This interpretation becomes even more stringent if the UV–VIS spectrum of the bis(tripeptide) (**11a**) [R = Pro-Val-(*R*)-Val-OMe] derived from the cyclic biliverdin (CBV) is considered (Table 3). In this compound the geometry is fixed by a four-link chain, and torsional angles can vary only within a narrow range.[11] Although compound (**11a**) contains the same peptidic chains as compound (**8**), its UV–VIS spectrum is almost superposable on that of the parent ester (**10**) [Figure 1(b)].

Two kinds of conformational change could be possible in these compounds. First, the small *f*-values might be due to an increase of the helical pitch of the bilatriene moiety. In this case the torsions around the C(5), C(10), and C(15) methine single bonds would exceed — at least partly — those found for the undisturbed geometry (*ca.* 15°)[1] but would remain less than 90°, thus preserving the all-*syn* conformation. Secondly, torsion angles might become larger than 90°, thus forming *anti* conformers ('stretched' species). The small variations in band position in compounds (**7**) and (**8**) when compared with the bands of compound (**1**) allow us to differentiate between these possibilities because λ_{max}-values of bilatrienes, in contrast to their absorptivities, are less governed by the conformer type (*syn* or *anti*) but mainly depend on deviations from coplanarity of the four heterocyclic rings.[12] In particular, the visible absorption band is known to experience a large shift if out-of-plane torsions occur. In essence, these theoretical predictions have been verified experimentally: if the pitch is decreased by insertion of a methano bridge between N(21) and N(24) the visible band undergoes a bathochromic shift by *ca.* 70 nm.[13] On the other hand for biliverdin-IXγ embedded in the bilin-binding protein from *Pierris brassicae* the pitch raises from 3.5 to 5.6 Å.[14] If compared with the urea-denatured biliverdin–protein complex a hypsochromic shift of *ca.* 35 nm takes place[15] while the dipole strength essentially remains unchanged.† Hence, the small *f*-values of compounds (**4**)–(**8**), (**14**), and (**15**) along with the substantial invariability of band positions*

* Clearly, the more pronounced shifts obtained for biliverdin bis(peptides) of sequence Pro-Leu-Y are due to the presence of two peptide chains and are no prerequisite for the small *f*-values observed. Deviations essentially vanish in the monopeptide (**14b**) (Δλ 4 nm) possessing a similar *f*-value (see below and Table 4).

† From data contained in ref. 15, absorptivities for the denatured biliverdin-IXγ–protein complex turn out to be approximately twice as large as those reported for biliverdin-IXγ.[2] Therefore, UV–VIS spectra of both the denatured and native biliprotein should be appropriately adapted.

Table 2. CD spectra [$\Delta\varepsilon_{max}$/dm^3 mol^{-1} cm^{-1} (λ_{max}/nm)] and molar rotations[a] $[M]^{20}_{546}$ of biliverdin bis- and mono-peptides (**2**)–(**9**), (**11**), and (**13**)–(**16**) in benzene.[b,c] The *s*-factor refers to the corresponding quotient of rotational strengths R_{UV}/R_{VIS}.[d]

Compound	Δε (λ)	*s*	$[M]^{20}_{546}$
(**2**)	+102.0 (650) −121.9 (380)	−1.2	−197 000
(**3**)	+98.0 (656) −110.0 (382)	−1.1	−202 000
(**4**)	+34.9 (662) −49.5 (382)	−1.5	−79 000
(**5**)	+59.4 (660) −82.7 (378)	−1.5	−123 000
(**6**)	+41.6 (659) −51.9 (381)	−1.5	−74 000
(**7**)	+49.6 (653) −66.0 (385)	−1.4	−95 000
(**8**)	+61.3 (655) −84.1 (384)	−1.4	−124 000
(**9**)	+105.6 (657) −144.1 (377)	−1.2	−225 000
(**11a**)[e]	+74.5 (648) −93.7 (379)	−1.2	−147 000
(**11b**)[e]	+43.3 (648) −51.9 (379)	−1.1	−84 000
(**13a**)[f]	+94.6 (652) −99.6 (378)	−1.1	−200 000
(**13b**)[f]	+11.5 (670) −2.4 (407) +2.2 (389) −5.7 (345)		−9 000
(**14a**)[f]	+44.0 (656) −56.3 (381)	−1.3	−87 000
(**14b**)[f]	−37.2 (645) +49.5 (385)	−1.3	+87 000
(**15a**)[f]	+52.0 (645) −61.0 (381)	−1.3	−119 000
(**15b**)[f]	−29.9 (645) +42.0 (384)	−1.4	+70 000
(**16a**)[f]	+94.6 (651) −115.7 (374)	−1.1	−209 000
(**16b**)[f]	+95.1 (655) −114.1 (373)	−1.1	−211 000

[a] ±5 000°. [b] See footnote *a* to Table 1. [c] CD spectra for solutions in chloroform and ethanol are given in the Experimental section. [d] For determination of rotational strengths, integration was performed from λ 800 nm to λ *ca.* 500 nm (visible band), and from λ *ca.* 450 nm (crossover point) to the crossover point at λ *ca.* 310 nm, which was extrapolated if hidden (UV band). [e] For structure, see Table 3. [f] For structure, see Table 4.

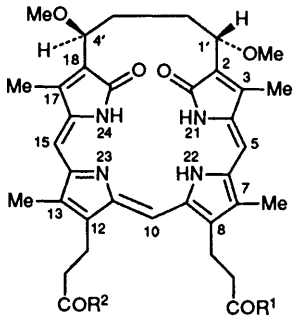
indicate the existence of stretched *anti* conformers, whose population decreases with increasing polarity of the solvent. If UV–VIS spectra of any of the biliverdin peptides (**4**)–(**8**), (**14**), and (**15**) are recorded in mixtures of the aprotic solvents benzene and chloroform in different proportions, then isosbestic points at λ 700 nm and λ *ca.* 400 nm appear. This is illustrated for the monopeptide (**15b**) for which differences in *f*-values between benzene and chloroform are largest (Figure 2). Except for the presence of *ca.* 10% of the rotamers which have a *cis* amide linkage between the propionic acyl residue and proline, ^{1}H NMR spectra in benzene and chloroform reveal homogeneity. For chloroform even down to 213 K no broadening of signals was observed, although the population of two bilatriene conformers must be assumed in this solvent. Thus, their mutual interconversion is rapid on the NMR time-scale.

The CD spectra of compounds (**4**)–(**8**) (Table 2) are too complex for any reliable conformational analysis to be made since neither the intrinsic CD parameters of any stretched species nor the extent of chiral induction for the coiled and stretched rotamers are known. Nevertheless, as can be seen from Table 2, a rough correlation between *f*-values (Table 1), *i.e.* stretching tendency, and the quotients of rotational strengths of the main CD bands, $s = R_{UV}/R_{VIS}$, exists. Accordingly, absolute *s*-values of compounds (**2**), (**3**), and (**9**) (s −1.1 to −1.2) adopting a helical conformation in benzene are smaller than those found for compounds (**4**)–(**8**) (s −1.4 to −1.5). This regularity also holds true if applied to compounds (**11**)–(**16**) (Table 2). However, *s* occasionally changes with the solvent without substantial changes of bilatriene conformation. Thus, the *s*-value of the helical, geometrically fixed compound (**11a**) varies from −1.2 (benzene) to −1.4 (ethanol). These results clearly demonstrate that any attempt to derive the conformation of bilatrienes from CD spectra alone would be problematic at least without appropriate reference compounds and under non-standardised conditions. Therefore the arguments in favour of a stretched conformation of the bilipeptides from *C*-phycocyanine in Tris buffer given in ref. 16 should be reconsidered. The conclusions rest upon the discrepancy between the quotient *s* of a helical biliverdin severely flattened by its N(21)–N(24) bridge [(s −0.33), this value is in misleading agreement with that computed by the semi-empirical method used (s − 0.36)], and those found for the chromopeptides studied (s −1.1 to −2.8).* From our study presented here it seems unlikely that a small peptide would be able to maintain a stretched bilatriene conformation in any protic medium (see below).

UV–VIS and CD Spectra of Biliverdin Mono(tripeptides) vs. *Bis(tripeptides)*.—Although the UV–VIS spectral data of compounds (**4**)–(**8**) impressively demonstrate the outstanding ability of the Pro-X-Y peptide sequence to change the conformation of the bilatriene backbone if covalently bound to the propionic side-chains, these compounds are less suited for a more detailed investigation of chromophore–peptide interactions or for the elucidation of a distinct conformational state. This is due to the presence of two peptide chains in close proximity which may give rise to interchain interactions.[7,8] This factor could be responsible for the stretching effects; thereby the two side-chains would act co-operatively, thus providing for the optimal spatial orientation to the chromophore. Alternatively, the conformational influence might arise from one single chain and the second entity would cause partial quenching of effects by non-bonded, interchain interactions. To check these possibilities a comparison of spectral properties of biliverdin mono- and bis-(peptides) was needed. We therefore synthesized some derivatives of the more symmetrical biliverdin-XIIIα isomer (BV-XIIIα) (**13**)–(**16**). As expected the two Pro-X-Y peptidic chains in compounds (**13a**), (**14a**), and (**15a**) exert a similar influence on *f*-values as found for the pertinent bis(peptides) in the BV-IXα series (**2**), (**6**), and (**8**): *f*-values of compounds (**14a**) and (**15a**) are significantly lower than those found for compound (**13a**) or for the parent ester (**12**) (Table 4). However, while the biliverdin mono- and bis-(peptides) of compounds (**14**) (R = Pro-Leu-Val-OMe) and (**15**) [R = Pro-Val-(*R*)-Val-OMe] show similar *f*-values differences for (**13**) (R = Pro-Ala-Ala-OMe) are strikingly large. These findings suggest that the susceptibility of UV–VIS spectra towards the bulkiness of the amino acid entity X as found for the BV-IXα (bis-peptides) (**2**)–(**8**) of sequence Pro-X-Y is mostly due to intramolecular

* UV–VIS spectra of these dihydrobiliverdin peptides can be expected to experience uncharacteristic changes if the dihydropyrrolone ring A is twisted around the C(5) methine single bond as has been claimed in ref. 16.

Table 3. UV–VIS spectra [ε_{max}/dm^3 mol^{-1} cm^{-1} (λ_{max}/nm)] of compound (**10**) and CBV bis- and mono-peptides (**11a**) and (**11b**) in various solvents.[a] The *f*-factor refers to the corresponding quotient of dipole strengths D_{UV}/D_{VIS}.[b,c]



		Benzene		Chloroform		Ethanol	
		ε (λ)	*f*	ε (λ)	*f*	ε (λ)	*f*
(**10**)	$R^1 = R^2$ = OMe	15 700 (658) 43 400 (384)	2.43	15 300 (660) 46 600 (384)	2.66	15 200 (661) 45 500 (380)	2.65
(**11a**)	$R^1 = R^2$ = Pro-Val-(*R*)-Val-OMe	14 300 (652) 39 000 (380)	2.44	14 200 (658) 45 500 (385)	2.80	14 100 (661) 44 800 (382)	2.73
(**11b**)	R^1 = Pro-Val-(*R*)-Val-OMe, R^2 = OMe	13 800 (656) 38 000 (381)	2.33	13 200 (659) 41 700 (384)	2.65	13 200 (660) 41 200 (381)	2.66

[a,b,c] See footnotes *a*, *b* and *c* to Table 1.

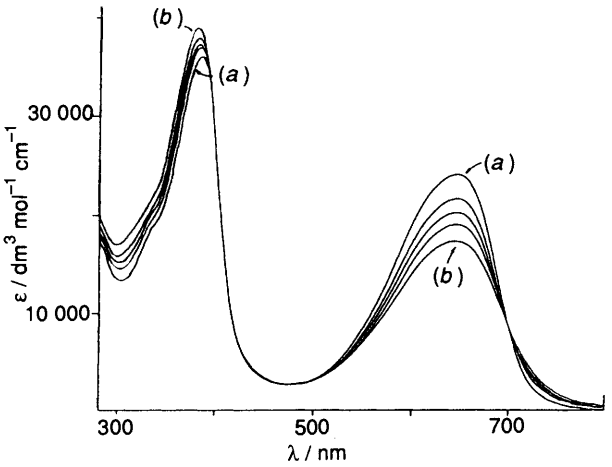


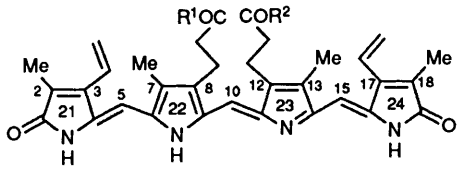
Figure 2. UV–VIS spectra of compound (**15b**) in benzene (*a*) and chloroform (*b*) and 2:1, 1:1, and 1:2 mixtures of these solvents for *ca.* 3×10^{-5} mol dm^{-3} solutions at 20 °C.

interchain interactions. Accordingly, competition with hydrogen bonds necessary to stretch the chromophore seems likely. These mutual interferences decrease as the bulkiness of X increases, are largest in compounds (**2**), (**3**), and (**13a**) (R = Pro-Ala-Y), and become low in compounds (**7**), (**8**), and (**15a**) (R = Pro-Val-Y). Nevertheless, a certain influence of the entity X remains even in the biliverdin monopeptides since *f* for compound (**13b**) is still significantly larger than *f* for compounds (**14b**) and (**15b**).

Additional valuable information can be obtained from a comparison of the corresponding CD spectra (Table 2). The intensity of the CD band centred around λ 660 nm of compound (**13b**) (R = Pro-Ala-Ala-OMe) in benzene is weakly positive but, surprisingly, the corresponding negative UV band is superimposed with a positive component (Table 2). For compounds (**14b**) and (**15b**), however, the visible band becomes negative and in the UV region only one positive band can be seen. These changes proceed with a concomitant increase in intensity of all CD bands. Hence the CD spectrum of the biliverdin monopeptide (**13b**) in benzene solution most likely comprises an envelope of two subspectra being of opposite rotational strengths with respect to the main bands. Since helical biliverdin peptides composed of (*S*)-α-amino acids at the relevant positions generally show a positive sign at λ *ca.* 660 nm and a negative sign at λ *ca.* 380 nm irrespective of whether either one or two peptide chains are present[7,8] the CD phenotype observed for the BV-XIIIα monopeptides (**14b**) and (**15b**) most probably reflects the property of the stretched species present in solution. Further examples of the CD characteristics of helical bilipeptides are supplied by the conformationally fixed derivatives (**11**) and by the flexible compounds (**16**) which reveal equal signs of the pertinent main CD bands independent of the number of peptide chains. Hence, the CD spectrum of compound (**13b**) indicates the coexistence of at least two species, one of which adopts a stretched conformation. Spectra of the bis(derivatives) (**4**)–(**8**), (**14a**), and (**15a**), however, do not allow any conclusion to be drawn with respect to the existence of non-helical conformers since the CD phenotype — despite the low *f*-values — strongly resembles that of (*Z,Z,Z,syn,syn,syn*) bilatrienes as (**2**), (**3**), (**9**), (**13a**), and (**16a**). The implication of this finding is discussed in more detail below.

Conformational Analysis by ^{1}H *NMR Spectroscopy.*—In order to elucidate more precisely the conformation of the stretched species of the biliverdin monopeptides (**13b**), (**14b**), and (**15b**) NOE experiments were performed. To probe the method for solutions in benzene on biliverdin peptides compound (**16b**) of known conformation (*Z,Z,Z,syn,syn,syn*) was used. The NOEs 5-H ⟶ 3-Vn-H_X (Vn = vinyl), 15-H ⟶ 17-Vn-H_X, 7-Me ⟶ 5-H, and 13-Me ⟶ 15-H confirm the *Z* configuration of the *exo* double bonds and *syn* conformation of the C(5) and C(15) single bonds. The resonance absorptions of the β-methylene protons of the propionic side-chains at C(8) and C(12) are poorly resolved and the signal enhancement seen comprises a superposition of two NOEs 8-CH_2 ⟶ 10-H and 12-CH_2 ⟶ 10-H. In chloroform the biliverdin monopeptides (**14b**) and (**15b**) still show all NOEs as found for peptide (**16b**) hence an appreciable amount of helical

Table 4. UV–VIS spectra [ε_{max}/dm^3 mol^{-1} cm^{-1} (λ_{max}/nm)] of compound (**12**) and BV-XIIIα bis- and mono-peptides (**13**)–(**16**) in various solvents.[a] The *f*-factor refers to the corresponding quotient of dipole strengths D_{UV}/D_{VIS}.[b,c]



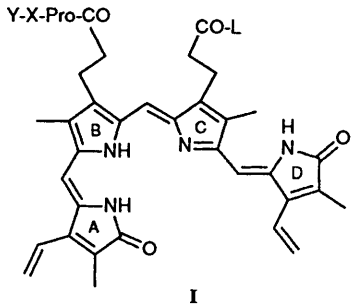
	Benzene		Chloroform		Ethanol	
	ε (λ)	*f*	ε (λ)	*f*	ε (λ)	*f*
(**12**) $R^1 = R^2$ = OMe	16 300 (651) 43 200 (380)	2.08	15 300 (651) 44 300 (377)	2.25	15 300 (656) 44 700 (376)	2.23
(**13a**) $R^1 = R^2$ = Pro-Ala-Ala-OMe	14 700 (648) 33 900 (381)	2.03	16 400 (647) 39 300 (380)	2.00	15 200 (650) 43 600 (376)	2.18
(**13b**) R^1 = Pro-Ala-Ala-OMe, R^2 = OMe	20 800 (645) 37 400 (381)	1.57	16 800 (646) 41 800 (378)	2.00	14 800 (653) 42 800 (376)	2.22
(**14a**) $R^1 = R^2$ = Pro-Leu-Val-OMe	21 600 (634) 33 100 (382)	1.41	19 400 (641) 37 600 (381)	1.59	15 700 (645) 41 400 (376)	2.05
(**14b**) R^1 = Pro-Leu-Val-OMe, R^2 = OMe	25 600 (647) 37 700 (384)	1.29	18 400 (644) 40 700 (379)	1.80	15 400 (647) 43 000 (376)	2.13
(**15a**) $R^1 = R^2$ = Pro-Val-(*R*)-Val-OMe	25 000 (647) 32 500 (385)	1.20	20 500 (644) 37 700 (381)	1.52	15 800 (643) 40 500 (377)	1.97
(**15b**) R^1 = Pro-Val-(*R*)-Val-OMe, R^2 = OMe	24 200 (648) 35 700 (383)	1.31	16 500 (644) 37 800 (378)	1.82	14 300 (646) 39 000 (376)	2.06
(**16a**) $R^1 = R^2$ = Ala-Pro-Ala-Ala-OMe	14 800 (649) 39 800 (377)	2.22	14 100 (652) 39 600 (376)	2.37	14 300 (656) 41 400 (377)	2.24
(**16b**) R^1 = Ala-Pro-Ala-Ala-OMe, R^2 = OMe	15 000 (654) 38 500 (377)	2.17	14 500 (657) 38 900 (377)	2.27	14 700 (655) 41 800 (376)	2.21

[a,b,c] See footnotes *a*, *b* and *c* to Table 1.

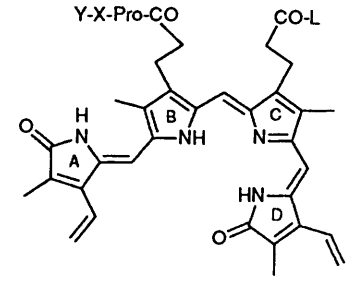
species must still be present. In benzene, however, all attempts to observe the NOE 7-Me ⟶ 5-H failed.* Bearing in mind that all other NOEs are observed and, considering the constitutional similarity with peptide (**16b**), the missing NOE in (**14b**) and (**15b**) becomes conformationally relevant, indicating rotation around the C(5) methine single bond. Whether additional rotation around the C(10) methine bond occurs cannot be assessed by this method alone (see above). However, if doubling in intensity of the visible absorption bands of compounds (**14b**) and (**15b**) would be due to additional rotation around the C(10) methine bond the UV bands should become exceptionally low, characteristic of an appreciably elongated bilatriene chromophore.[2,14,17]

Two isomers **I** and **II** (Scheme, L = OMe) are possible for the stretched species deduced for the biliverdin-XIIIα monopeptides (**14b**) and (**15b**) in benzene. The results of the NOE experiments described above indicate that this equilibrium is largely shifted to one side. However, from our experiments alone we cannot decide whether conformer **I** or **II** predominates.

The similarity of UV–VIS spectra of the monopeptides (**14b**) and (**15b**) with the corresponding bis(peptides) (**14a**) and (**15a**) also suggests similar conformations of stretched species despite their antipodal CD spectra. However, the two conformers **I** and **II** (Scheme, L = Pro-X-Y) become identical in the biliverdin-XIIIα series [(**14a**) and (**15a**)] and should be nearly equipopulated in the biliverdin-IXα series [(**4**)–(**8**)], in which only the substituents located on ring D are interchanged. Consequently for biliverdin bis(peptides) NOE experiments do



I



II

Scheme.

* It is not possible to distinguish between 5-H and 15-H. Here and throughout the numbering scheme refers to conformer **II** displayed in the Scheme (L = OMe) in accord with the mechanism of conformational changes proposed (see below).

not allow us to differentiate between a helical conformation and rapidly interconverting stretched species **I** ⇌ **II**. Hence the NOEs 7-Me ⟶ 5-H and 13-Me ⟶ 15-H observed for compounds (**6**) (R = Pro-Leu-Val-OMe) and (**8**) [R = Pro-

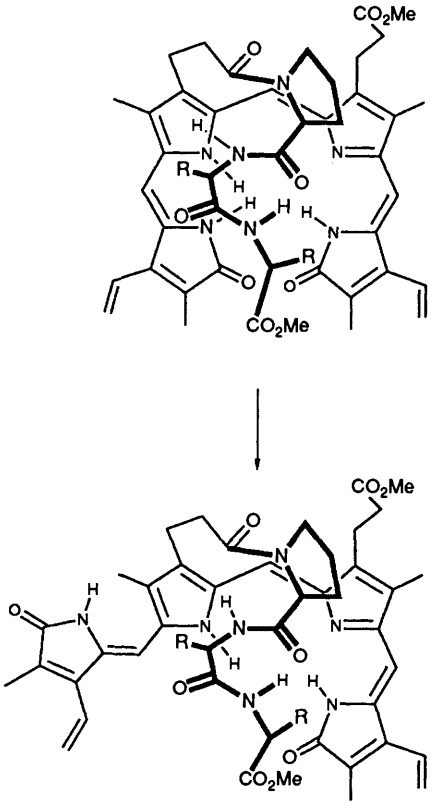


Figure 3. Model design illustrating the conformational changes from the (*Z,Z,Z,syn,syn,syn*)to the *Z,Z,Z,anti,syn,syn*) geometry suggested for flexible biliverdin monopeptides of sequence Pro-X-Y.

Val-(*R*)-Val-OMe] with comparable intensities do not conflict with an eventual (*Z,Z,Z,anti,syn,syn*) conformation but are insufficient to exclude other possibilities, *e.g.* the coexistence of (*Z,Z,Z,syn,syn,syn*) with minor amounts of (*Z,Z,Z,anti,syn,anti*) conformers. If any conformational similarity between the stretched species of mono- and bis-(peptide) derivatives exists, differences in CD spectra, might be due to a reversal of chiral discrimination between antipodal stretched bilatriene species caused by the influence of the second peptide entity. Alternatively, conformational differences might be restricted to one torsional angle increasing over the others or changing its sign without affecting the (*Z,Z,Z,anti,syn,syn*) arrangement.

Conformation-determining Chromophore–Peptide Interactions.—A model to rationalise the outstanding conformational influence of Pro-X-Y peptides on the flexible bilatriene backbone must include the following findings: (i) Proline at the *N*-terminus comprises an intrinsic constituent. The sequence Pro-X alone, however, does not give rise to conformational changes but the third amino acid Y is a necessary prerequisite for chromophore stretching. Thus, if biliverdin-IXα is bound to the sequence Pro-Leu-OMe or Leu-Leu-OMe, the *f*-values (*f* 2.47 and 2.41, respectively)[18] indicate preservation of the conformation in contrast to the homologue (**5**) (R = Pro-Leu-Leu-OMe) (*f* 1.70) (Table 1). If a leucyl entity is inserted between the propionic side-chain and the prolyl moiety of compound (**5**), thus affording compound (**9**) (R = Leu-Pro-Leu-Leu-OMe) (*f* 2.75), recoiling takes place. (ii) The chromophore-stretching ability of the tripeptide Pro-X-Y markedly decreases if the polarity of the solvent is increased, thus accounting for the appreciable solvatochromism of bilipeptides (**4**)–(**8**), (**13b**), (**14**), and (**15**). (iii) Efficient hydrogen bonding between the 2*H*-pyrrole nitrogen [N(23)] and the hydrogens located at the pyrrole and pyrrolinone nitrogens mainly accounts for the general preference of the (*Z,Z,Z,syn,syn,syn*) conformation of bilatrienes.[1] Therefore in any stretched conformation this energetically favoured arrangement must be disrupted and appropriate compensation provided by interactions with the peptide chain. This can only take place if the peptide hydrogen-bonding-donor and -acceptor sites adopt an optimised spatial relation to the congruent sites of the bilatriene backbone.

From the large number of small peptides investigated with respect to secondary structures, proline has been shown to favour the formation of bends easily detectable by CD spectroscopy.[19] Unfortunately the peptide absorption region below λ *ca.* 230 nm is not accessible in chloroform solutions, and in apolar solvents such as cyclohexane biliverdin peptides are insoluble. Besides, interferences with CD bands stemming from the bilatriene moiety can be expected to occur. The secondary structure of the Pro-X-Y peptides is corroborated by CD investigations on the corresponding *N*-acetyl-protected derivatives. They exhibit a similar proportion of the *trans*-acyl-prolyl rotamer (*ca.* 90%, chloroform and benzene) as found for the related biliverdin peptides with the exception of compounds (**11**) (40–60%) in which the formation of this rotamer seems to be hindered due to the rigidity of the helical bilatriene moiety. In cyclohexane, molar ellipticities at λ *ca.* 228 nm are in the range of −25 000 to −45 000, indicating the existence of a γ-turn secondary structure[19,20] in apolar solvents which in ethanol [θ(λ 225 nm) −3 000 to −6 000] becomes largely destroyed.

A γ-bend formed between the propionic carbonyl and the amide hydrogen of the second amino acid entity X allows the N–H bond of the *C*-terminal amino acid Y to become situated along an axis parallel to the direction of the pyrrolinone N–H bond of ring A of the bilatriene moiety, as shown in the model design (Figure 3). Both the proximity and direction of the N–H bonds to the 2*H*-pyrrole nitrogen acceptor site results in efficient competition. Thus, the equilibrium is shifted in favour of the stretched species of type **II** (*cf.* Scheme, L = OMe) by rotation around the C(5) methine single bond (Figure 3). Formation of γ-turns is mainly restricted to poor hydrogen-bonding solvents and may easily be disturbed by other proximate peptides,[19] in agreement with the pronounced dependence of the stretching tendency from solvents and the influence of a second peptide chain. However, hydrogen bonding with the solvent not only influences the secondary structure of the peptide but also serves as a potential intermolecular competitor with the peptide–2*H*-pyrrole hydrogen bond. This influence additionally contributes to weakening of the relevant chromophore–peptide interactions favouring recoiling of the bilatriene backbone. Owing to their large hydrophobic groups valyl and leucyl entities exert additional torsional constraints on the peptide entity. In consequence, the spatial orientation of the hydrogen bond between the *C*-terminal amino acid and the 2*H*-pyrrole nitrogen is further restricted, and efficiency of interaction is optimised. In view of these considerations the increase in stretching tendency of the bilatriene moiety on going from biliverdin peptide (**13b**) (R = Pro-Ala-Ala-OMe) to compounds (**14b**) (R = Pro-Leu-Val-OMe) and (**15b**) [R = Pro-Val-(*R*)-Val-OMe] becomes plausible.

Conclusions

Up to now only covalently bound native proteins rather than peptides have been shown to change the energetically favourable (*Z,Z,Z,syn,syn,syn*) conformation of bilatrienes. Thus, in *C*-phycocyanine, torsion around the C(5) and C(15) methine single bonds occurs. Thereby a large number of hydrogen bonds and salt linkages between the bilatriene moiety and the apoprotein are responsible for the stretching of the

chromophore.[21] Our study reveals evidence that even small peptides may give rise to conformational changes if the sequence is appropriately chosen, although the potential interaction sites of a tripeptide are strongly limited *per se*. This limitation accounts for the fact that the conformational changes observed with the biliverdin peptides (**4**)–(**8**), (**13b**), (**14**), and (**15**) are essentially restricted to poorly hydrogen-bonding solvents.

Experimental

M.p.s were determined with a Kofler-Reichert hot-stage apparatus. ^{1}H NMR spectra were recorded at 250 MHz or at 400 MHz with Bruker instruments (WM 250, AM 400) at 297 K if not stated otherwise for solutions in [$^2H_6$]benzene and $CDCl_3$ (both chromatographed on alumina prior to use). NOE difference spectra were obtained for *ca.* 5 × 10^{-3} mol dm^{-3} solutions deaerated by three freeze-pump-thaw cycles. Molecular masses have been determined by fast-atom bombardment mass spectrometry (FABMS) using a Finnigan MAT 900 (glycerol; Xe). UV–VIS spectra were measured with a Perkin-Elmer Lambda 7 spectrometer (0.1–10 cm quartz cuvettes) equipped with a data station 3 600. CD spectra were taken with a Jobin Yvon Mark III instrument carrying cylindrical quartz cuvettes (0.01–10 cm). Optical rotations (10 cm path length) were obtained with a Perkin-Elmer 241 instrument. All optical measurements were carried out in thermostatted cell compartments (20 ± 1 °C). As solvents, spectroscopic grade benzene, methanol, ethanol (all Uvasol, Merck), and chloroform (Lichrosolv, Merck) were used. Benzene and chloroform were chromatographed on alumina prior to use. To prevent interferences with protonated species due to traces of acids occasionally present in the solvents, triethylamine (1 ppm) was routinely added to solutions prior to measurements in benzene or chloroform. This additive does not influence spectra. Column chromatography was performed on silica gel (Kieselgel 60, 230–400 mesh, Merck). Chloroform (Lichrosolv, Merck) used for elution was purified with alumina prior to use. The purity (> 95%) of biliverdin peptides prepared was assessed by ^{1}H NMR and TLC (Kieselgel 60 F_{254}, 0.25 mm, Merck; $CHCl_3$–MeOH 97:3 to 90:10 v/v). All synthetic procedures were carried out under an inert atmosphere (Ar) and protected from light.

Starting Materials.—Biliverdin-IXα (BV-IXα), biliverdin-XIIIα (BV-XIIIα), and the cyclic biliverdin (CBV)—a mixture of chiral, thermally interconvertible diastereoisomers of [(*P*,1′*R*,4′*R*) + (*M*,1′*S*,4′*S*)]- and [(*M*,1′*R*,4′*R*) + (*P*,1′*S*,4′*S*)]-2,18-(1′,4′-dimethoxybutane-1′,4′-diyl)-8,12-bis-(2″-hydroxycarbonylethyl)-3,7,13,17-tetramethyl-1,19-(21*H*,24*H*)-bilindione—were obtained by saponification of the parent esters (**1**),[22] (**12**),[22] and (**10**),[11] respectively. The peptide esters used for the preparation of the biliverdin derivatives (**2**)–(**9**), (**11**), and (**13**)–(**16**) were synthesized in a stepwise manner by the phosphorazo and/or hydroxysuccinimide method[23] starting from the *C*-terminal amino acid ester. The intermediate and final deprotections of benzyloxycarbonyl compounds were performed by hydrogenolysis over Pd in methanol containing hydrogen chloride (1 mol equiv.). Their diastereoisomeric homogeneity (94–98%) was assessed by GLC[24] after hydrolysis with HCl (6 mol dm^{-3}) for 24 h at 110 °C in sealed ampoules.

Syntheses of BV-IXα Bis(peptides) (**2**)–(**9**).—These compounds were prepared according to the general procedure given in ref. 8, starting from BV-IXα, the appropriate peptide ester hydrochloride, and *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodi-imide hydrochloride (Sigma). After column chromatography ($CHCl_3$–MeOH 93:7 v/v) the main fraction was triturated with benzene–hexane and centrifuged. The supernatant was discarded and the remaining dark powder was dried *in vacuo* (0.1 mmHg; 50 °C). This afforded compounds (**2**)–(**9**) (yield 40–60%).

Compound (**2**) (R = Pro-Ala-Ala-OMe) was prepared from (−)-H-Pro-Ala-Ala-OMe·HCl {$[\alpha]_D^{20}$ −100.7° (*c* 1, MeOH)}; no m.p., gradually decomposing on heating up to 300 °C; *m/z* 1 089 (M^+ + H); CD λ_{max}($CHCl_3$) 660 (Δε +34.0 dm^3 mol^{-1} cm^{-1}) and 380 nm (−41.9); λ_{max}(EtOH) 658 (Δε +9.6 dm^3 mol^{-1} cm^{-1}) and 378 nm (−14.1); for the CD spectrum in benzene, molar rotation, UV–VIS spectra, and the ^{1}H NMR spectrum see Tables 1, 2, and 5.

Compound (**3**) (R = Pro-Ala-Val-OMe) was prepared from (−)-H-Pro-Ala-Val-OMe·HCl {$[\alpha]_D^{20}$ −80.6° (*c* 2, MeOH)}; m.p. 240–250 °C (decomp.); *m/z* 1 145.5 (M^+ + H); CD λ_{max}($CHCl_3$) 662 (Δε +27.0) and 381 nm (−30.6); λ_{max}(EtOH) 665 (Δε +9.5) and 377 nm (−12.3); for the CD spectrum in benzene, molar rotation, UV–VIS spectra, and the ^{1}H NMR spectrum see Tables 1, 2, and 5.

Compound (**4**) (R = Pro-Leu-Gly-OEt) was prepared from (−)-H-Pro-Leu-Gly-OEt·AcOH {$[\alpha]_D^{20}$ −77.6° (*c* 3, EtOH)}; m.p. 196–200 °C; *m/z* 1 173.5 (M^+ + H); CD λ_{max}($CHCl_3$) 663 (Δε +20.0) and 380 nm (−40.1); λ_{max}(EtOH) 665 (Δε +10.8) and 377 nm (−24.1); for the CD spectrum in benzene, molar rotation, UV–VIS spectra, and the ^{1}H NMR spectrum see Tables 1, 2, and 5.

Compound (**5**) (R = Pro-Leu-Leu-OMe) was prepared from (−)-H-Pro-Leu-Leu-OMe·HCl {$[\alpha]_D^{20}$ −72.0° (*c* 2, MeOH)}; m.p. 215–220 °C; *m/z* 1 258.0 (M^+ + H); CD ($CHCl_3$) within the range λ 800–300 nm |Δε| < 7 dm^3 mol^{-1} cm^{-1}; λ_{max}(EtOH) 670 (Δε +9.4) and 377 nm (−13.0); for the CD spectrum in benzene, molar rotation, UV–VIS spectra, and the ^{1}H NMR spectrum see Tables 1, 2, and 5.

Compound (**6**) (R = Pro-Leu-Val-OMe) was prepared from (−)-H-Pro-Leu-Val-OMe·HCl {$[\alpha]_D^{20}$ −67.9° (*c* 1, MeOH)}; m.p. 185–187 °C; *m/z* 1 229.5 (M^+ + H); CD ($CHCl_3$, EtOH) within the range λ 800–300 nm |Δε| < 7 dm^3 mol^{-1} cm^{-1}; for the CD spectrum in benzene, molar rotation, UV–VIS spectra, and the ^{1}H NMR spectrum see Tables 1, 2, and 5.

Compound (**7**) (R = Pro-Val-Val-OMe) was prepared from (−)-H-Pro-Val-Val-OMe·HCl {$[\alpha]_D^{20}$ −72.1° (*c* 2, MeOH)}; m.p. 220–223 °C; *m/z* 1 201.0 (M^+ + H); CD λ_{max}($CHCl_3$) 662 (Δε +9.1) and 382 nm (−15.5); λ_{max}(EtOH) 665 (Δε +6.2) and 377 nm (−12.0); for the CD spectrum in benzene, molar rotation, UV–VIS spectra, and the ^{1}H NMR spectrum see Tables 1, 2, and 5.

Compound (**8**) [R = Pro-Val-(*R*)-Val-OMe] was prepared from (−)-H-Pro-Val-(*R*)-Val-OMe·HCl {$[\alpha]_D^{20}$ −36.0° (*c* 2, MeOH)}; m.p. 210–213 °C; *m/z* 1 201.5 (M^+ + H); CD λ_{max}($CHCl_3$) 652 (Δε +15.9) and 380 nm (−24.9); λ_{max}(EtOH) 670 (Δε +7.6) and 377 nm (−12.0); for the CD spectrum in benzene, molar rotation, UV–VIS spectra, and ^{1}H NMR spectra see Tables 1, 2, and 5.

Compound (**9**) (R = Leu-Pro-Leu-Leu-OMe) was prepared from (−)-H-Leu-Pro-Leu-Leu-OMe·HCl {$[\alpha]_D^{20}$ −87.1° (*c* 2, MeOH)}; m.p. 135–140 °C; *m/z* 1 484.0 (M^+ + H); CD λ_{max}($CHCl_3$) 658 (Δε +100.7) and 377 nm (−144.9); λ_{max}(EtOH) 663 (Δε +72.5) and 376 nm (−91.6); for the CD spectra in benzene, molar rotation, UV–VIS spectra, and the ^{1}H NMR spectra see Tables 1, 2, and 5.

Syntheses of CBV- and BV-XIIIα Mono- and Bis-(peptides).—General procedure. To a stirred solution of the appropriate biliverdin [CBV (45 mg) or BV-XIIIα (41 mg) (0.07 mmol)] and the peptide ester hydrochloride (0.21 mmol) in tetrahydrofuran (THF) (5 cm^3)–water (1 cm^3) cooled to 0 °C were added successively *N*-[3-dimethylamino)propyl]–*N*′-ethylcarbodi-imide hydrochloride (130 mg, 0.7 mmol) and pyridine (17 mm^3, 0.21 mmol). Reaction was continued until the ratio of bis(peptide) and mono(peptide) (as the half-acid) was *ca.* 1:2

(1–2 h) by TLC control, $CHCl_3$–MeOH 93:7 v/v, R_F(mono) < R_F(bis)]. Then MeOH (0.4 cm^3) and further carbodiimide (*ca.* 100 mg, 0.5 mmol) were added. The solution was kept in a refrigerator overnight, poured into $CHCl_3$ (25 cm^3), and stripped twice with hydrochloric acid (0.001 mol dm^{-3}; 2 × 25 cm^3), aq. sodium hydrogen carbonate, and water. The organic layer was dried (sodium sulphate) and the solvent was evaporated off under reduced pressure. The residue was chromatographed on silica gel (column 25 × 2 cm). Gradient elution with $CHCl_3$ containing 5–10% v/v MeOH afforded the esters (**10**) or (**12**) (0.015 mmol), biliverdin mono(peptides) (*ca.* 0.02 mmol), and biliverdin bis(peptides) (*ca.* 0.02 mmol) in that order. Trituration of the glassy fractions with benzene–hexane mixtures, centrifugation, and drying (0.1 mmHg; 50 °C) afforded the pure chromopeptides, overall yield 50–80%. Products (**11a**) and (**11b**) consist of two diastereoisomers rapidly interconverting at ambient temperature (*cf.* ref. 11).

Table 5. ^{1}H NMR spectra[a,b] of bilipeptides (**2**)–(**9**) and (**13**)–(**16**)[c] and their assignment.[d]

Compound	Biliverdin moiety	Acyl substituent R
(**2**)[e]	6.92 (1 H, s, 10-H), 6.66 (1 H, m, 3-Vn-H_X), 6.52, 6.16, and 5.44 (1 H × 3, XMA, J_{XM} 18, J_{XA} 11.5, J_{MA} 2 Hz, 18-Vn), 6.13 and 6.10 (1 H × 2, each s, 5- and 15-H), 5.67 (2 H, m, 3-Vn-H_{AB}), 2.94 (4 H, m, 8- and 12-CH_2), 2.7–2.3 (4 H, m, CH_2CO), 2.21 (3 H, s, 17-Me), 2.13 and 2.11 (3 H × 2, each s, 7- and 13-Me), and 1.90 (3 H, s, 2-Me)	7.56 (1 H, d, *J* 7.0 Hz, NH), 7.44 (1 H, d, *J* 6.8 Hz, NH), 7.11 (1 H, d, *J* 7.5 Hz, NH), 6.98 (1 H, d, *J* 7.0 Hz, NH), *ca.* 4.4 (6 H, m, C_α-H), 3.69 (6 H, s, CO_2Me), 3.7–3.3 (4 H, m, Pro C_δ-H), 2.3–1.7 (8 H, m, Pro C_β- and C_γ-H), and *ca.* 1.3 (12 H, m, Ala-Me)
(**3**)[e]	6.93 (1 H, s, 10-H), 6.66 (1 H, m, 3-Vn-H_X), 6.51, 6.18, and 5.43 (1 H × 3, XMA, J_{XM} 17.2, J_{XA} 11.4, J_{MA} 2 Hz, 18-Vn), 6.13 and 6.11 (1 H × 2, each s, 5- and 15-H), 5.67 (2 H, m, 3-Vn-H_{AB}), 2.95 (4 H, m, 8- and 12-CH_2), 2.6–2.2 (4 H, m, CH_2CO), 2.21 (3 H, s, 17-Me), 2.13 and 2.11 (3 H × 2, each s, 7- and 13-Me), and 1.90 (3 H, s, 2-Me)	7.40 (1 H, d, *J* 7.5 Hz, NH), *ca.* 7.25 (1 H, m, NH), 7.13 (1 H, d, *J* 8.0 Hz, NH), 7.00 (1 H, d, *J* 7.8 Hz, NH), 4.42 (6 H, m, C_α-H), 3.69 (6 H, s, CO_2Me), 3.6–3.2 (4 H, m, Pro C_δ-H), 2.2–1.7 (10 H, m, Pro C_β- and C_γ-H, Val C_β-H), 1.28 and 1.20 (3 H × 2, each d, *J* 7.0 Hz, Ala-Me), and *ca.* 0.85 (12 H, m, Val-Me)
(**4**)[e]	6.93 (1 H, s, 10-H), 6.65 (1 H, m, 3-Vn-H_X), 6.51, 6.18, and 5.45 (1 H × 3, XMA, J_{XM} 18.0, J_{XA} 11.5, J_{MA} 2 Hz, 18-Vn), 6.08 and 6.03 (1 H × 2, each s, 5- and 15-H), 5.66 (2 H, m, 3-Vn-H_{AB}), *ca.* 2.9 (4 H, m, 8- and 12-CH_2), 2.63 (4 H, m, CH_2CO), 2.19 (3 H, s, 17-Me), 2.08 and 2.06 (3 H × 2, each s, 7- and 13-Me), and 1.92 (3 H, s, 2-Me)	7.46 (1 H, t, *J* 5.5 Hz, NH), 7.37 (1 H, t, *J* 5.5 Hz, NH), 7.26 (1 H, d, *J* 8.4 Hz, NH), 7.19 (1 H, d, *J* 8.5 Hz, NH), 4.55 and 4.34 (2 H × 2, m, Pro and Leu C_α-H), 4.06 and 3.87 (2 H × 2, m, Gly C_α-H), 4.12 (4 H, q, *J* 7.5 Hz, CO_2CH_2), 3.56 and 3.22 (2 H × 2, m, Pro C_δ-H), 2.2–1.5 (14 H, m, Pro and Leu C_β- and C_γ-H), 1.22 (6 H, t, *J* 7.5 Hz, CO_2CH_2*Me*), 0.86 and 0.84 (6 H × 2, d, *J* 6 Hz, Leu-Me)
(**5**)[e]	6.83 (1 H, s, 10-H), 6.68 (1 H, m, 3-Vn-H_X), 6.53, 6.25, and 5.45 (1 H × 3, XMA, J_{XM} 18.0, J_{XA} 11.3, J_{MA} 2 Hz, 18-Vn), 6.12 and 6.10 (1 H × 2, each s, 5- and 15-H), 5.67 (2 H, m, 3-Vn-H_{AB}), 2.95 (4 H, m, 8- and 12-CH_2), *ca.* 2.6 (4 H, m, CH_2CO), 2.22 (3 H, s, 17-Me), 2.09 and 2.08 (3 H × 2, each s, 7- and 13-Me), and 1.97 (3 H, s, 2-Me)	7.47 (1 H, d, *J* 7.5 Hz, NH), 7.17 (1 H, d, *J* 7.5 Hz, NH), 7.05 (1 H, d, *J* 7.8 Hz, NH), 6.88 (1 H, d, *J* 7.5 Hz, NH), 4.45 (6 H, m, C_α-H), 3.68 (6 H, s, CO_2Me), 3.6–3.2 (4 H, m, Pro C_δ-H), 2.2–1.6 (20 H, m, Pro and Leu C_β- and C_γ-H), and *ca.* 0.85 (24 H, m, Leu-Me)
(**6**)[e]	6.84 (1 H, s, 10-H), 6.68 (1 H, m, 3-Vn-H_X), 6.53, 6.26, and 5.44 (1 H × 3, XMA, J_{XM} 17.5, J_{XA} 11.5, J_{MA} 2 Hz, 18-Vn), 6.12 and 6.09 (1 H × 2, each s, 5- and 15-H), 5.67 (2 H, m, 3-Vn-H_{AB}), 2.95 (4 H, m, 8- and 12-CH_2), 2.7–2.5 (4 H, m, CH_2CO), 2.22 (3 H, s, 17-Me), 2.08 (6 H, s, 7- and 13-Me), and 1.97 (3 H, s, 2-Me)	*ca.* 7.3 (1 H, m, NH), 7.16 (1 H, d, *J* 7.5 Hz, NH), 7.09 (1 H, d, *J* 8.2 Hz, NH), 7.02 (1 H, d, *J* 8.2 Hz, NH), *ca.* 4.45 (6 H, m, C_α-H), 3.70 (6 H, s, CO_2Me), 3.6–3.2 (4 H, m, Pro C_δ-H), 2.2–1.6 (16 H, m, Pro and Leu C_β- and C_γ-H, Val C_β-H), and 0.85 (24 H, m, Leu-Me, Val-Me)
(**7**)[e]	6.84 (1 H, s, 10-H), 6.71 (1 H, m, 3-Vn-H_X), 6.55, 6.30, and 5.45 (1 H × 3, XMA, J_{XM} 18.0, J_{XA} 11.0, J_{MA} 2 Hz, 18-Vn), 6.14 and 6.13 (1 H × 2, each s, 5- and 15-H), 5.67 (2 H, m, 3-Vn-H_{AB}), 2.96 (4 H, m, 8- and 12-CH_2), 2.64 (4 H, m, CH_2CO), 2.24 (3 H, s, 17-Me), 2.11 and 2.10 (3 H × 2, each s, 7- and 13-Me), and 2.00 (3 H, s, 2-Me)	7.40 (1 H, d, *J* 9.0 Hz, NH), 7.3–7.2 (2 H, m, NH), 7.16 (1 H, d, *J* 8.5 Hz, NH), 4.5–4.2 (6 H, m, C_α-H), 3.71 (6 H, s, CO_2Me), 3.44 and 3.27 (2 H × 2, m, Pro C_δ-H), 2.3–1.7 (12 H, m, Pro C_β- and C_γ-H, Val C_β-H), and 0.90 (24 H, m, Val-Me)
(**8**)[e]	6.85 (1 H, s, 10-H), 6.71 (1 H, m, 3-Vn-H_X), 6.54, 6.28, and 5.44 (1 H × 3, XMA, J_{XM} 17.5, J_{XA} 11.0, J_{MA} 2 Hz, 18-Vn), 6.14 and 6.12 (1 H × 2, each s, 5- and 15-H), 5.68 (2 H, m, 3-Vn-H_{AB}), 2.95 (4 H, m, 8- and 12-CH_2), 2.7–2.5 (4 H, m, CH_2CO), 2.23 (3 H, s, 17-Me), 2.12 and 2.10 (3 H × 2, each s, 7- and 13-Me), and 1.99 (3 H, s, 2-Me)	7.33 (1 H, d, *J* 7.0 Hz, NH), 7.20 (1 H, d, *J* 7.0 Hz, NH), 7.14 (1 H, d, *J* 8.0 Hz, NH), 6.91 (1 H, d, *J* 8.0 Hz, NH), 4.5–4.3 (6 H, m, C_α-H), 3.68 and 3.67 (3 H × 2, each s, CO_2Me), 3.48 and 3.25 (2 H × 2, m, Pro C_δ-H), 2.3–1.7 (12 H, m, Pro C_β- and C_γ-H, Val C_β-H), and *ca.* 0.9 (24 H, m, Val-Me)
(**8**)[e,f]	6.98 (1 H, s, 10-H), 6.61 (1 H, m, 3-Vn-H_X), *ca.* 6.4 (2 H, m, 18-Vn-H_{XY}), 6.14 (1 H, s, 5-H), 6.10 (1 H, s, 15-H), 5.48 (2 H, m, 3-Vn-H_{AB}), 5.36 (1 H, m, 18-Vn-H_A), 2.9–2.4 (8 H, m, 8- and 12-CH_2CH_2CO), 2.06 (3 H, s, 13-Me), 2.02 (3 H, s, 17-Me), 1.98 (3 H, s, 7-Me), and 1.88 (3 H, s, 2-Me)	7.70, 7.57, 7.38, and *ca.* 7.2 (1 H × 4, each d, NH), 4.55 (6 H, m, C_α-H), 3.39 and 3.38 (3 H × 2, each s, CO_2Me), *ca.* 3.3 (4 H, m, Pro C_δ-H), 2.2–1.7 (12 H, m, Pro C_β- and C_γ-H, Val C_β-H), and *ca.* 0.8 (24 H, m, Val-Me)
(**9**)[e]	6.61 (1 H, m, 3-Vn-H_X), 6.54 (1 H, s, 10-H), 6.50, 6.15, and 5.45 (1 H × 3, XMA, J_{XM} 18.0, J_{XA} 12.0, J_{MA} 2 Hz, 18-Vn), 6.08 and 6.04 (1 H × 2, each s, 5- and 15-H), 5.66 (2 H, m, 3-Vn-H_{AB}), 3.1–2.3 (8 H, m, 8- and 12-CH_2CH_2CO), 2.19 (3 H, s, 17-Me), 2.10 and 2.07 (3 H × 2, each s, 7- and 13-Me), and 1.86 (3 H, s, 2-Me)	7.77 and 7.70 (1 H × 2, each d, *J* 7.5 Hz, NH), 6.7–6.4 (2 H, m, NH), 6.35 (2 H, d, *J* 8.0 Hz, NH), 4.7–4.2 (8 H, m, C_α-H), 3.68 (6 H, s, CO_2Me), 3.6–3.3 (4 H, m, Pro C_δ-H), 2.3–1.4 (26 H, m, Pro and Leu C_β- and C_γ-H), and *ca.* 0.9 (36 H, m, Leu-Me)
(**13a**)[e,g]	6.88 (1 H, s, 10-H), 6.67 (2 H, m, 3- and 17-Vn-H_X), 6.10 (2 H, s, 5- and 15-H), 5.70 and 5.67 (2 H × 2, m, 3- and 17-Vn-H_{AB}), *ca.* 2.95 (4 H, m, 8- and 12-CH_2), 2.8–2.5 (4 H, m, CH_2CO), 2.10 (6 H, s, 7- and 13-Me), and 1.94 (6 H, s, 2- and 18-Me)	7.43 (2 H, d, *J* 7.2 Hz, NH), 7.09 (2 H, d, *J* 7.3 Hz, NH), 4.46 (2 H, m, Pro C_α-H), 4.43 and 4.40 (2 H × 2, m, Ala C_α-H), 3.67 (6 H, s, CO_2Me), 3.56 and 3.34 (2 H × 2, m, Pro C-H), *ca.* 2.0 (8 H, m, Pro C_β- and C_γ-H), 1.37 and 1.28 (6 H × 2, d, *J* 7.2 Hz, Ala-Me)
(**13b**)[g,h]	6.80 (1 H, s, 10-H), 6.65 and 6.62 (1 H × 2, m, 3- and 17-Vn-H_X), 6.07 and 6.03 (1 H × 2, each s, 5- and 15-H), *ca.* 5.7 (4 H, m, 3- and 17-Vn-H_{AB}), *ca.* 2.95 (4 H, m, 8- and 12-CH_2), *ca.* 2.6 (4 H, m, CH_2CO), 2.07 (6 H, s, 7- and 13-Me), and 1.94 (6 H, s, 2- and 18-Me)	7.09 (1 H, d, *J* 7.6 Hz, NH), 7.05 (1 H, d, *J* 7.4 Hz, NH), 4.51 and 4.49 (1 H × 2, quin, *J* 7.5 Hz, Ala C_α-H), 4.44 (1 H, m, Pro C_α-H), 3.73 and 3.65 (3 H × 2, s, CO_2Me), 3.47 and 3.32 (1 H × 2, m, Pro C_δ-H), *ca.* 2.0 (4 H, m, Pro C_β- and C_γ-H), 1.41 and 1.36 (3 H × 2, d, *J* 7.5 Hz, Ala-Me)

Table 5 (*continued*).

Compound	Biliverdin moiety	Acyl substituent R
(14a)[e]	6.80 (1 H, s, 10-H), 6.67 (2 H, m, 3- and 17-Vn-H_X), 6.10 (2 H, s, 5- and 15-H), 5.66 (4 H, m, 3- and 17-Vn-H_{AB}), 2.94 (4 H, m, 8- and 12-CH_2), 2.60 (4 H, m, CH_2CO), 2.06 (6 H, s, 7- and 13-Me), and 1.98 (6 H, s, 2- and 18-Me)	7.24 (2 H, d, *J* 8.5 Hz, NH), 7.10 (2 H, d, *J* 8.0 Hz, NH), 4.45 (6 H, m, C_α-H), 3.69 (6 H, s, CO_2Me), 3.43 and 3.28 (2 H × 2, m, Pro C_δ-H), 2.2–1.6 (16 H, m, Pro and Leu C_β- and C_γ-H, Val C_β-H), and *ca.* 0.9 (24 H, m, Leu-Me, Val-Me)
(14b)[h]	6.78 (1 H, s, 10-H), 6.67 and 6.60 (1 H × 2, m, 3- and 17-Vn-H_X), 6.10 and 6.02 (1 H × 2, each s, 5- and 15-H), 5.67 (4 H, m, 3- and 17-Vn-H_{AB}), 2.96 and 2.90 (2 H × 2, m, 8- and 12-CH_2), 2.58 (4 H, m, CH_2CO), 2.05 and 2.04 (3 H × 2, each s, 7- and 13-Me), 1.96 and 1.94 (3 H × 2, each s, 2- and 18-Me)	7.16 (1 H, d, *J* 8.4 Hz, NH), 6.98 (1 H, d, *J* 8.0 Hz, NH), 4.6–4.4 (3 H, m, C_α-H), 3.70 and 3.63 (3 H × 2, s, CO_2Me), *ca.* 3.3 (2 H, m, Pro C_δ-H), 2.2–1.5 (8 H, m, Pro and Leu C_β- and C_γ-H, Val C_β-H), and *ca.* 0.9 (12 H, m, Leu-Me, Val-Me)
(14b)[h,i]	7.09 (1 H, s, 10-H), 6.87 (1 H, m, 3-Vn-H_X), 6.31, 5.38, and 5.26 (1 H × 3, XBA, J_{XB} 17.6, J_{XA} 11.7, J_{BA} 1.5 Hz, 17-Vn), 6.26 (1 H, s, 5-H), 5.91 (1 H, s, 15-H), *ca.* 5.5 (2 H, m, 3-Vn-H_{AB}), 2.86 (4 H, m, 8- and 12-CH_2), *ca.* 2.5 (4 H, m, CH_2CO), 2.09 (3 H, s, 7-Me), 1.90 and 1.87 (3 H × 2, each s, 2- and 18-Me), and 1.81 (3 H, s, 13-Me)	8.22 (1 H, d, *J* 8.0 Hz, Val NH), 6.74 (1 H, d, *J* 9.3 Hz, Leu NH), *ca.* 5.3 (1 H, m, Leu C_α-H), 4.78 (1 H, dd, J_1 8.0, J_2 5.4 Hz, Val C_α-H), 4.12 (1 H, dd, J_1 7.6, J_2 3.0 Hz, Pro C_α-H), 3.33 and 3.29 (3 H × 2, each s, CO_2Me), 3.3–2.9 (2 H, m, Pro C_δ-H), 2.4–1.3 (8 H, m, Pro and Leu C_β- and C_γ-H, Val C_β-H), and *ca.* 1.0 (12 H, m, Leu-Me, Val-Me)
(15a)[e]	6.83 (1 H, s, 10-H), 6.70 (2 H, m, 3-and 17-Vn-H_X), 6.12 (2 H, s, 5- and 15-H), 5.68 (4 H, m, 3- and 17-Vn-H_{AB}), 2.94 (4 H, m, 8- and 12-CH_2), 2.63 (4 H, m, CH_2CO), 2.09 (6 H, s, 7- and 13-Me), and 2.00 (6 H, s, 2- and 18-Me)	7.28 (2 H, d, *J* 8.9 Hz, NH), 7.05 (2 H, d, *J* 8.6 Hz, NH), 4.5–4.3 (6 H, m, C_α-H), 3.67 (6 H, s, CO_2Me), 3.48 and 3.26 (2 H × 2, m, Pro C_δ-H), 2.2–1.7 (12 H, m, Pro C_β- and C_γ-H, Val C_β-H), and *ca.* 0.9 (24 H, m, Val-Me)
(15b)[h]	6.79 (1 H, s, 10-H), 6.66 and 6.60 (1 H × 2, m, 3- and 17-Vn-H_X), 6.08 and 6.02 (1 H × 2, each s, 5- and 15-H), 5.67 (4 H, m, 3- and 17-Vn-H_{AB}), 2.96 and 2.90 (2 H × 2, m, 8- and 12-CH_2), 2.57 (4 H, m, CH_2CO), 2.05 and 2.04 (3 H × 2, each s, 7- and 13-Me), 1.95 and 1.94 (3 H × 2, each s, 2- and 18-Me)	7.03 (1 H, d, *J* 8.2 Hz, NH), 6.96 (1 H, d, *J* 8.5 Hz, NH), 4.45 (3 H, m, C_α-H), 3.67 and 3.62 (3 H × 2, each s, CO_2Me), 3.42 and 3.32 (1 H × 2, m, Pro C_δ-H), 2.3–1.8 (6 H, m, Pro C_β- and C_γ-H, Val C_β-H), and 0.94 (12 H, m, Val-Me)
(15b)[h,i]	7.10 (1 H, s, 10-H), 6.83 (1 H, m, 3-Vn-H_X), 6.31, 5.37, and 5.26 (1 H × 3, XBA, J_{XB} 17.6, J_{XA} 11.8, J_{BA} 1.5 Hz, 17-Vn), 6.25 (1 H, s, 5-H), 5.91 (1 H, s, 15-H), *ca.* 5.5 (2 H, m, 3-Vn-H_{AB}), 2.87 (4 H, m, 8- and 12-CH_2), 2.7–2.4 (4 H, m, CH_2CO), 2.08 (3 H, s, 7-Me), 1.88 and 1.86 (3 H × 2, each s, 2- and 18-Me), and 1.81 (3 H, s, 13-Me)	8.11 (1 H, d, *J* 8.0 Hz, NH), 6.67 (1 H, d, *J* 9.6 Hz, NH), 5.11 (1 H, dd, J_1 9.6, J_2 8.0 Hz, Val C_α-H), 4.78 (1 H, dd, J_1 8.0, J_2 7.0 Hz, Val C_α-H), 4.10 (1 H, dd, J_1 7.6, J_2 3.4 Hz, Pro C_α-H), 3.30 and 3.24 (3 H × 2, each s, CO_2Me), 3.4–2.9 (2 H, m, Pro C_δ-H), 2.4–1.8 (6 H, m, Pro C_β- and C_γ-H, Val C_β-H), 1.20, 1.17, 1.04, and 1.02 (3 H × 4, each d, *J* 7.0 Hz, Val-Me)
(16a)[e]	6.62 (2 H, m, 3- and 17-Vn-H_X), 6.46 (1 H, s, 10-H), 6.06 (2 H, s, 5- and 15-H), 5.67 (4 H, m, 3- and 17-Vn-H_{AB}), 3.2–2.5 (8 H, m, 8- and 12-CH_2CH_2CO), 2.04 (6 H, s, 7- and 13-Me), and 1.91 (6 H, s, 2- and 18-Me)	7.76 (2 H, d, *J* 6.7 Hz, NH), 6.76 (2 H, d, *J* 7.7 Hz, NH), 6.34 (2 H, d, *J* 7.5 Hz, NH), *ca.* 4.5 (6 H, m, C_α-H), 4.23 (2 H, quin, *J ca.* 7 Hz, Ala C_α-H), 3.69 (6 H, s, CO_2Me), *ca.* 3.7 and 3.45 (2 H × 2, m, Pro C_δ-H), 2.1–1.7 (8 H, m, Pro C_β- and C_γ-H), 1.34 (6 H, d, *J* 7.0 Hz, Ala-Me), and 1.22 (12 H, d, *J* 7.0 Hz, Ala-Me)
(16b)	6.63 and 6.57 (1 H × 2, m, 3- and 17-Vn-H_X), 6.50 (1 H, s, 10-H), 6.14 and 5.91 (1 H × 2, s, 5- and 15-H), *ca.* 5.7 (4 H, m, 3- and 17-Vn-H_{AB}), 2.9–2.6 (8 H, m, 8- and 12-CH_2CH_2CO), 2.05 and 2.01 (3 H × 2, each s, 7- and 13-Me), 1.92 and 1.89 (3 H × 2, each s, 2- and 18-Me)	8.23 (1 H, d, *J* 6.0 Hz, NH), 6.83 (1 H, d, *J* 7.0 Hz, NH), 5.77 (1 H, d, *J* 7.5 Hz, NH), 4.63 (1 H, m, Pro C_α-H), 4.47, 4.44, and 4.17 (1 H × 3, quin, *J ca.* 7 Hz, Ala C_α-H), 3.67 and 3.65 (3 H × 2, each s, CO_2Me), 3.7–3.3 (2 H, m, Pro C_δ-H), 2.0–1.6 (4 H, m, Pro C_β- and C_γ-H), 1.32, 1.20, and 1.17 (3 H × 3, each d, *J* 7.0 Hz, Ala-Me)
(16b)[i]	6.77 (1 H, s, 10-H), 6.44, 5.55, and 5.23 (1 H × 3, XBA, J_{XB} 17.5, J_{XA} 11.0, J_{BA} 1.5 Hz, 3- or 17-Vn), 6.25 (1 H, m, 17- or 3-Vn-H_X), 5.95 and 5.80 (1 H × 2, each s, 5- and 15-H), *ca.* 5.3 (2 H, m, 17- or 3-Vn-H_{AB}), 3.0–2.3 (8 H, m, 8- and 12-CH_2CH_2CO), 1.97 (3 H, s, 18- or 2-Me), 1.78 (3 H, s, 13- or 7-Me), 1.72 (3 H, s, 2- or 18-Me), and 1.66 (3 H, s, 7- or 13-Me)	8.62 (1 H, d, *J* 6.0 Hz, NH), 6.95 (1 H, d, *J* 7.7 Hz, NH), 5.67 (1 H, d, *J* 8.5 Hz, NH), 5.04 (1 H, dd, J_1 7.0, J_2 3.0 Hz, Pro C_α-H), 4.70, 4.59, and 4.45 (1 H × 3, m, Ala C_α-H), 3.33 and 3.17 (3 H × 2, each s, CO_2Me), 3.3–2.9 (2 H, m, Pro C_δ-H), 2.2–1.5 (4 H, m, Pro C_β- and C_γ-H), 1.30, 1.28, and 1.12 (3 H × 3, each d, *J* 7.0 Hz, Ala-Me)

[a] Chemical shifts are given in δ(ppm) downfield from $SiMe_4$ in *ca.* 10^{-2} mol dm^{-3} solutions. [b] 250 MHz, 297 K, in $CDCl_3$. [c] For ^1H NMR spectra of the macrocycles (**11a**) and (**11b**) see Experimental section. [d] Most of the assignments could simply be established by drawing a comparison with the corresponding biliverdin esters [(**1**) and (**12**)], peptide esters, and *N*-benzyloxycarbonyl peptide esters, and were complemented by double resonance and NOE experiments. [e] Main rotamer (> 70%) *trans,trans* with respect to the propionic–proline amide bonds. The portion of the *trans,cis* and *cis,cis* rotamers (< 30%) could not be determined with sufficient accuracy due to severe overlapping of resonance absorptions. [f] In [$^2H_6$]benzene–$CDCl_3$ 2:1 v/v 308 K. [g] 400 MHz. [h] Main rotamer (> 90%) *trans* with respect to the propionic–proline amide bond. [i] In [$^2H_6$]benzene, 308 K.

CBV-bis(peptide) (**11a**) [$R^1 = R^2 =$ *Pro-Val-*(R)*-Val-OMe*] *and CBV-monopeptide* (**11b**) [$R^1 =$ *Pro-Val-*(R)*-Val-OMe*, $R^2 =$ *OMe*].—These compounds were prepared from (−)-H-Pro-Val-(*R*)-Val-OMe·HCl.

Compound (**11a**) had m.p. 146–156 °C; *m/z* 1 263.5 (M^+ + H); δ_H(400 MHz; $CDCl_3$) major diastereoisomer [(*P*,1′*R*,4′*R*) + (*M*,1′*S*,4′*S*)] (72%) [mixture of three acyl-prolyl rotamers (*cis,trans*), (*trans,trans*), and (*cis,cis*) in the proportions 50:35(or 15):15(or 35) corresponding to a participation of the *trans*-rotamer of *ca.* 60% (or 40%)] biliverdin moiety: 11.59, 11.56, and 11.55 (1 H, br, s, 22-/23-H), *ca.* 7.3 (1 H, s, 10-H), 6.9–6.7 (2 H, br s, 21- and 24-H), 6.41, 6.40, and 6.38 (2 H, s, 5- and 15-H), 4.08 (2 H, d, *J* 12.0 Hz, 1′- and 4′-H), *ca.* 3.23 (6 H, s, 1′- and 4′-OMe), 3.2–3.0 (4 H, m, 8- and 12-CH_2), 2.66 (4 H, m, CH_2CO), 2.4–1.8 (4 H, m, 2′-and 3′-H_2), 2.33 (6 H, s, 7- and 13-Me), 2.25 and 2.24 (6 H, s, 3- and 17-Me), acyl substituents: *ca.* 7.3 (2 H, m, NH), 6.77 (2 H, d, *J* 8.4 Hz, NH), 4.6–4.2 (6 H, m, C_α-H), 3.74–3.69 (6 H, s, CO_2Me), 3.6–3.3 (4 H, m, Pro C_δ-H), 2.3–1.8 (12 H, m, Pro C_β- and C_γ-H, Val C_β-H), and *ca.* 0.9 (24 H, m, Val-Me); minor diastereoisomer [(*M*,1′*R*,4′*R*) + (*P*,1′*S*,4′*S*)] (28%) [mixture of three acyl-prolyl rotamers (*cis,trans*, *trans,trans*, and *cis,cis*) in the proportions 50:35(or 15):15(or 35) corresponding to a participation of the *trans*-rotamer of 60% (or 40%)] biliverdin moiety: 12.6 and 12.5 (1 H, br s, 22-/23-H), 7.4–7.1 (2 H, br s, 21- and 24-H), *ca.* 7.15 (1 H, s, 10-H), 6.28, 6.27, and 6.26 (2 H, s, 5- and 15-H), *ca.* 4.5 (2 H, br s, 1′-and 4′-H), *ca.* 3.35 (6 H, s, 1′-and 4′-OMe), 3.2–3.0 (4 H, m, 8- and 12-CH_2), 2.66 (4 H, m, CH_2CO), 2.4–1.8 (4 H, m, 2′- and 3′-H_2), 2.39 and 2.38 (6 H, s, 7-and 13-

Me), 2.19 and 2.17 (6 H, s, 3- and 17-Me), acyl substituents: *ca.* 7.3 (2 H, m, NH), 7.0–6.8 (2 H, m, NH), 4.6–4.2 (6 H, m, C_α-H), 3.74–3.69 (6 H, s, CO_2Me), 3.6–3.3 (4 H, m, Pro C_δ-H), 2.3–1.8 (12 H, m, Pro C_β- and C_γ-H, Val C_β-H), and *ca.* 0.9 (24 H, m, Val-Me); CD λ_{max}($CHCl_3$) 650 ($\Delta\varepsilon$ +20.9) and 378 nm (−33.2); λ_{max}(EtOH) 652 ($\Delta\varepsilon$ +23.0) and 376 nm (−36.0); for the CD spectrum in benzene, molar rotation, and UV–VIS spectra see Tables 2 and 3.

Compound (**11b**) had m.p. 142–150 °C; *m/z* 968 (M^+ + H); δ_H(250 MHz; $CDCl_3$) major diastereoisomer [(*P*,1′*R*,4′*R*) + (*M*,1′*S*,4′*S*)] (78%) (mixture of two acyl-prolyl rotamers *trans* and *cis* in the ratio 60:40 or 40:60) biliverdin moiety: 11.58 (1 H, br s, 22-/23-H), 7.22 (1 H, s, 10-H), 6.8–6.7 (2 H, br s, 21- and 24-H), *ca.* 6.4 (2 H, s, 5- and 15-H), 4.07 (2 H, d, *J* 10.0 Hz, 1′- and 4′-H), 3.22 (6 H, s, 1′-and 4′-OMe), *ca.* 3.05 (4 H, m, 8- and 12-CH_2), *ca.* 2.65 (4 H, m, CH_2CO), 2.37 and 2.23 (6 H × 2, each s, 3-, 7-, 13-, and 17-Me), and 2.3–1.8 (4 H, m, 2′- and 3′-H_2), acyl substituents: 7.3–7.2 (1 H, m, NH), 6.8–6.6 (1 H, d, *J ca.* 6 Hz, NH), 4.5–4.3 (3 H, m, C_α-H), *ca.* 3.7 (3 H, s, CO_2Me), *ca.* 3.6 (3 H, s, CO_2Me), 3.7–3.4 (2 H, m, Pro C_δ-H), 2.3–1.8 (6 H, m, Pro C_β- and C_γ-H, Val C_β-H), and *ca.* 0.9 (12 H, m, Val-Me); minor diastereoisomer [(*M*,1′*R*,4′*R*) + (*P*,1′*S*,4′*S*)] (22%) (mixture of two acyl-prolyl rotamers *trans* and *cis* in the ratio 60:40 or 40:60) biliverdin moiety: *ca.* 12.5 (1 H, br s, 22-/23-H), *ca.* 7.25 (2 H, br s, 21- and 24-H), 7.08 (1 H, s, 10-H), *ca.* 6.25 (2 H, s, 5- and 15-H), *ca.* 4.6 (2 H, br s, 1′- and 4′-H), *ca.* 3.3 (6 H, s, 1′- and 4′-OMe), *ca.* 3.0 (4 H, m, 8- and 12-CH_2), *ca.* 2.6 (4 H, m, CH_2CO), 2.38 (6 H, s, 7- and 13-Me), 2.3–1.8 (4 H, m, 2′- and 3′-H_2), and 2.17 (6 H, s, 3- and 17-Me), acyl substituents: 7.3–7.2 (1 H, m, NH), 6.7–6.6 (1 H, d, *J ca.* 8 Hz, NH), 4.5–4.2 (3 H, m, C_α-H), 3.68 and 3.65 (3 H × 2, each s, CO_2Me), 3.7–3.4 (2 H, m, Pro C_δ-H), 2.3–1.8 (6 H, m, Pro C_β- and C_γ-H, Val C_β-H), and *ca.* 0.9 (12 H, m, Val-Me); CD ($CHCl_3$) within the range λ 800–300 nm $|\Delta\varepsilon| < 7$ dm^3 mol^{-1} cm^{-1}; λ_{max}(EtOH) 650 ($\Delta\varepsilon$ + 7.7) and 380 nm (−9.6); for the CD spectrum in benzene, molar rotation, and UV–VIS spectra see Tables 2 and 3.

BV-XIIIα Bis(peptide) (**13a**) (R^1 = R^2 = *Pro-Ala-Ala-OMe*) *and BV-XIIIα Monopeptide* (**13b**) (R^1 = *Pro-Ala-Ala-OMe*, R^2 = *OMe*).—These compounds were prepared from (−)-H-Pro-Ala-Ala-OMe·HCl. Compound (**13a**) had no m.p., gradually decomposing on heating up to 300 °C; *m/z* 1 089 (M^+ + H); CD λ_{max}($CHCl_3$) 655 ($\Delta\varepsilon$ +33.0) and 380 nm (−30.5); λ_{max}(EtOH) 655 ($\Delta\varepsilon$ +6.0) and 375 nm (−8.3); for the CD spectrum in benzene, molar rotation, UV–VIS spectra, and the ^{1}H NMR spectrum see Tables 2, 4, and 5.

Compound (**13b**) had m.p. 239–241 °C; *m/z* 850 (M^+ + H); CD ($CHCl_3$, EtOH) within the range λ 800–300 nm $|\Delta\varepsilon| < 7$ dm^3 mol^{-1} cm^{-1}; for the CD spectrum in benzene, molar rotation, UV–VIS spectra, and the ^{1}H NMR spectrum see Tables 2, 4, and 5.

BV-XIIIα Bis(peptide) (**14a**) (R^1 = R^2 = *Pro-Leu-Val-OMe*) *and BV-XIIIα Monopeptide* (**14b**) (R^1 = *Pro-Leu-Val-OMe*, R^2 = *OMe*).—These compounds were prepared from (−)-H-Pro-Leu-Val-OMe·HCl. Compound (**14a**) had m.p. 220–222 °C; *m/z* 1 229.5 (M^+ + H); CD ($CHCl_3$, EtOH) within the range λ 800–300 nm $|\Delta\varepsilon| < 7$ dm^3 mol^{-1} cm^{-1}; for the CD spectrum in benzene, molar rotation, UV–VIS spectra, and the ^{1}H NMR spectrum see Tables 2, 4, and 5.

Compound (**14b**) had m.p. 186–189 °C; *m/z* 920.0 (M^+ + H); CD λ_{max}($CHCl_3$) 655 ($\Delta\varepsilon$ −11.6) and 385 nm (+14.2); CD (EtOH) within the range λ 800–300 nm $|\Delta\varepsilon| < 7$ dm^3 mol^{-1} cm^{-1}; for the CD spectrum in benzene, molar rotation, UV–VIS spectra, and ^{1}H NMR spectra see Tables 2, 4, and 5.

BV-XIIIα Bis(peptide) (**15a**) [R^1 = R^2 = *Pro-Val*-(R)-*Val-OMe*] *and BV-XIIIα Monopeptide* (**15b**) [R^1 = *Pro-Val*-(R)-*Val-OMe*, R^2 = *OMe*].—These compounds were prepared from (−)-H-Pro-Val-(*R*)-Val-OMe·HCl.

Compound (**15a**) had m.p. 238–242 °C (decomp.); *m/z* 1 201.5 (M^+ + H); CD λ_{max}($CHCl_3$) 648 ($\Delta\varepsilon$ +11.9) and 380 nm (−16.0); CD (EtOH) within the range λ 800–300 nm $|\Delta\varepsilon| < 7$ dm^3 mol^{-1} cm^{-1}; for the CD spectrum in benzene molar rotation, UV–VIS spectra, and the ^{1}H NMR spectrum see Tables 2, 4, and 5.

Compound (**15b**) had m.p. 197–199 °C; *m/z* 906.0 (M^+ + H); CD λ_{max}($CHCl_3$) 645 ($\Delta\varepsilon$ −9.2) and 383 nm (+14.4); CD (EtOH) within the range λ 800–300 nm $|\Delta\varepsilon| < 7$ dm^3 mol^{-1} cm^{-1}; for the CD spectrum in benzene, molar rotation, UV–VIS spectra, and ^{1}H NMR spectra see Tables 2, 4, and 5.

BV-XIIIα Bis(peptide) (**16a**) (R^1 = R^2 = *Ala-Pro-Ala-Ala-OMe*), *and BV-XIIIα Monopeptide* (**16b**) (R^1 = *Ala-Pro-Ala-Ala-OMe*, R^2 = *OMe*).—These compounds were prepared from (−)-H-Ala-Pro-Ala-Ala-OMe·HCl {$[\alpha]_D^{20}$ −136.5° (*c* 1, MeOH)}. Compound (**16a**) had m.p. 237–240 °C (decomp.); *m/z* 1 231.5 (M^+ + H); CD λ_{max}($CHCl_3$) 652 ($\Delta\varepsilon$ +110.8) and 374 nm (−130.0); λ_{max}(EtOH) 656 ($\Delta\varepsilon$ +38.6) and 377 nm (−46.8); for the CD spectrum in benzene, molar rotation, UV–VIS spectra, and the ^{1}H NMR spectrum see Tables 2, 4, and 5.

Compound (**16b**) had m.p. 214–217 °C; *m/z* 921.0 (M^+ + H); CD λ_{max}($CHCl_3$) 657 ($\Delta\varepsilon$ +95.1) and 372 nm (−111.2); λ_{max}(EtOH) 658 ($\Delta\varepsilon$ +22.4) and 376 nm (−25.9); for the CD spectrum in benzene, molar rotation, UV–VIS spectra, and ^{1}H NMR spectra see Tables 2, 4, and 5.

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