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Conformation Control of Bilatrienes by Peptides: A Survey**

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Summary. A variety of bilipeptides has been synthesized and investigated by UV-VIS and CD spectroscopy to explore the conformational influence of peptide ligands on the covalently bound tetrapyrrol moiety. The results obtained are complemented by those reported previously. From the comprehensive data thus available several features of the peptidic chain regulating interactions with the bilatriene moiety have been elucidated. Accordingly, the presence of three amide linkages at the ligand is prerequiste for induction of pronounced conformational changes consisting either in a complete transition of *M* into *P* helical bilatriene species by inversion of all torsional angles, or in a stretching of the chromophore. The kind of conformational influence can be controlled by varying the position of the prolyl entity within a given peptide.

Keywords. Bilipeptides; Conformation control; Chiral discrimination; Bilatrienes, non-helical.

Konformative Kontrolle von Bilatrienen durch Peptide: Ein Überblick

Zusammenfassung. Eine gezielte Auswahl von Bilipeptiden wurde dargestellt; ihre UV-VIS und CD-Spektren wurden bezüglich einer konformativen Einflußnahme auf die kovalent gebundenen Tetrapyrrol-Einheiten untersucht und die Ergebnise durch kürzlich veröffentlichte ergänzt. Aus den so zugänglichen umfangreichen Daten lassen sich einige Charakteristika in der Bauweise der Peptidketten herleiten, die die Wechselwirkung mit dem Bilatrienteil bestimmen. Es zeigt sich, daß der Ligand zumindest drei Amidbindungen aufweisen muß, um ausgeprägte Konformationsänderungen hervorzurufen. Diese Einflußnahme äußert sich entweder in einem vollständigen Übergang von *M* in *P* helikale Bilatrien-Spezies und kommt durch gleichzeitige Inversion aller Torsionswinkel zustande, oder aber sie bewirkt eine Streckung des Bilatrienteils. Die Art der Konformationsänderung läßt sich durch Variation der Position einer Prolyleinheit innerhalb eines vorgegebenen Peptides lenken.

Introduction

The conformation control of co-factors by proteins is one of the most intriguing fields in bioorganic chemistry. Thus, the singular photophysical properties of the tetrapyrrolic moieties in biliproteins are due to non-bonding interactions with the proteins by stabilizing one distinct geometrical arrangement over numerous others along with a concomitant enhancement of rigidity [1]. However, the regularities governing the mutual influence of co-factor and apoprotein have been less inves-

** Dedicated to Prof. Dr. Kurt Schaffner on occasion of his 60th birthday

tigated until now. We therefore have initiated a systematic study on that subject [2–7], which is aimed at providing a better understanding of the efficacy of conformation determining hydrogen bonding sites and, in a second approach, assessing conditions to simulate the conformational changes in native biliproteins.

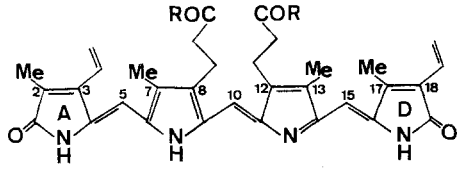
In a previous report [5] we have demonstrated that even small peptides may affect the conformation of the highly flexible bilatriene backbone. The study presented now enlargens and complements recent investigations in this field dealing with the efficacy of peptide ligands of varying size, chain length, and sequence.

Results and Discussion

General Considerations

In Tables 1–3 the *f*-values of compounds **1–5**, **7–23**, and **25–30** listed refer to the quotients of dipol strengths D_{UV}/D_{VIS} of the first UV and VIS absorption bands centered around λ ca. 380 nm and λ ca. 660 nm, respectively. They must be compared with that of biliverdin-IX α dimethyl ester (**6**) (bilipeptides **1–5**, **7–23**) and in the XIII α series (bilipeptides **25–30**), with the ester **24**; **6**, and **24** thus serve as conformational standards. *f*-Values of biliverdin esters adopting a helical (*Z, Z, Z, syn, syn, syn*) geometry in usual solvents amount to 2.50 ± 0.25 (**6**) and 2.15 ± 0.20 (**24**), respectively, making allowance for fluctuations within 10% [8]. Deviations from these ranges indicate deviations from the standard helix geometry. If *f*-values of bilipeptides are close to that of **6** or **24** [9], respecively, CD spectra reflect the extent of chiral discrimination between *M* and *P* helical species. Based on a Δε value of ca. +110 (chloroform) and ca. +100 (chloroform-methanol 98 : 2 *v/v*, benzene, ethanol) for the visible band (λ ca. 660 nm) of the bilatriene *P* helix [4] the discrimination constant $K = x_P/x_M$ (Tables 1–3) may directly be estimated. *f*-values smaller than those of the standards **6** and **24** indicate the presence of stretched

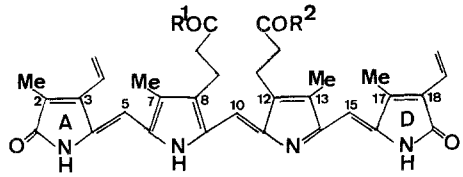
Table 1. Ratio *f* of dipole strengths D_{UV}/D_{VIS} and equilibrium constant *K* for chiral discrimination between *M* and *P* bilatriene helices. Values refer to chloroform-methanol (98 : 2 *v/v*) of ca. $3 \cdot 10^{-5}$ mol dm^{-3} solutions at 20°C[a]



Compound	*R*	*f*	*K*
1	Ala-Ala-O*Me*	2.40	1.5
2	Ala-Ala-NH*Me*	2.76	39
3	Ala-Ala-Gly-O*Me*	2.35	> 50
4	Ala-Ala-Ala-O*Me*	2.62	> 50
5	Ala-Ala-Ala-Ala-O*Me*	2.70	> 50

[a] Obtained from UV-VIS and CD spectra contained in Tables 4 and 5, and Ref. [4]. For determination of *f* see Ref. [5]. Configurations of amino acids refer to (*S*)-chirality

Table 2. Ratio f of dipole strengths D_{UV}/D_{VIS} and equilibrium constant K for chiral discrimination between M and P bilatriene helices for biliverdin-IXα derivatives in various solvents of ca. $3 \cdot 10^{-5}$ mol dm^{-3} solutions at 20°C [a]



Compound	$R^1 = R^2$	Benzene		Chloroform		Ethanol	
		f	K	f	K	f	K
6	O*Me*	2.40	1	2.62	1	2.62	1
7	Leu-Gly-Pro-O*Me*	2.43	> 50	2.58	9.4	2.51	2.8
8	Val-Val-Pro-O*Me*	2.11	[b]	2.57	1.4	2.56	1.3
9	Ala-Pro-Ala-Ala-O*Me*	2.61	> 50	2.68	> 50	2.69	3.1
10	Ala-Pro-Ala-Val-O*Me*	2.65	> 50	2.77	> 50	2.78	3.1
11	Val-Pro-Ala-Val-O*Me*	2.76	> 50	2.73	> 50	2.72	3.2
12	Leu-Pro-Leu-Leu-O*Me*	2.75	> 50	2.76	23	2.78	6.3
13	Pro-Ala-Ala-O*Me*	2.42	> 50	2.32	1.9	2.58	1.2
14	Pro-Leu-Gly-O*Et*	1.68	[b]	2.10	[b]	2.57	1.2
15	Pro-Val-Val-O*Me*	1.47	[b]	1.72	[b]	2.33	1.1
16	Pro-Val-(*R*)-Val-O*Me*	1.37	[b]	1.69	[b]	2.26	1.2
17	Pro-Leu-Leu-O*Me*	1.70	[b]	1.76	[b]	2.30	1.2
18	Pro-Leu-O*Me*	2.47	1.1	2.72	1.1	2.74	1.0
19	Leu-Leu-O*Me*	2.41	1.3	2.63	1.3	2.57	1.3
20	Ala-Ala-Pro-Ala-Ala-O*Me*	[c]	[c]	2.33	3.8	2.51	1.4
21	Val-Val-Pro-Ala-Ala-O*Me*	2.15	[b]	2.60	ca. 1	2.64	1.4
22	Ala-Val-Pro-Ala-Val-O*Me*	1.81	[b]	2.22	1.8	2.61	1.2
	R^1 (R^2 = O*Me*)						
23[d]	Ala-Val-Pro-Ala-Val-O*Me*	2.28	1.6	2.66	1.2	2.70	1.0

[a] Obtained from UV-VIS and CD spectra contained in Tables 4 and 5, and Ref. [5]. For determination of f see Ref. [5]. Configurations of amino acids refer to (*S*)-chirality if not stated otherwise

[b] Deviation from standard geometry

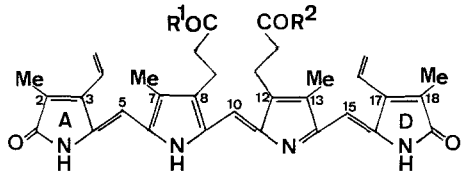
[c] Insoluble in benzene

[d] Isomeric assignment arbitrary; R^1 and R^2 in **23**, obtained as a single compound, may be interchanged

bilatriene species [10]. Since their fractional population, chiroptical properties, and extent of chiral discrimination are unknown, corresponding CD spectra are less informative and K-values cannot be determined as indicated in Tables 2 and 3.

The following discussion is mostly devoted to chloroform solutions since regularities are most pronounced in this solvent. Due to the a priori larger peptide chromophor interactions in benzene, minute differences in peptidic chains affecting conformation often become obscured.

Table 3. Ratio f of dipole strengths D_{UV}/D_{VIS} and equilibrium constant K for chiral discrimination between M and P bilatriene helices for biliverdin-XIIIα derivatives in various solvents of ca. $3 \cdot 10^{-5}$ mol dm^{-3} solutions at 20°C [a]



Compound	$R^1 = R^2$	Benzene		Chloroform		Ethanol	
		f	K	f	K	f	K
24	O*Me*	2.08	1	2.25	1	2.23	1
25	Pro-Ala-Ala-O*Me*	2.03	> 50	2.00	1.9	2.18	1.1
26	Pro-Val-(*R*)-Val-O*Me*	1.20	[b]	1.52	[b]	1.97	ca. 1
27	Leu-Gly-Pro-O*Me*	2.11	> 50	2.20	8.0	2.12	2.4
	R^1 (R^2 = O*Me*)						
28	Pro-Ala-Ala-O*Me*	1.57	[b]	2.00	1.0	2.22	1.0
29	Pro-Val-(*R*)-Val-O*Me*	1.31	[b]	1.82	[b]	2.06	1.0
30	Leu-Gly-Pro-O*Me*	2.16	> 50	2.17	7.8	2.15	2.9

[a] Obtained from UV-VIS and CD spectra contained in Tables 4 and 5, and Ref. [5]. For determination of f see Ref. [5]. Configurations of amino acids refer to (*S*)-chirality if not stated otherwise

[b] Deviation from standard geometry

Conformation Control

The f-values of compounds **1–5** ranging from $f = 2.35$ to $f = 2.76$ indicate an undisturbed helical conformation of the corresponding tetrapyrrolic moieties. By comparison of the peptidic entities of bilipeptides **1–5** (Table 1) it follows that the discriminating efficiency observed for **1** (K being as low as 1.5, as generally observed with dipeptide ligands) can be appreciably increased if the terminal ester grouping is simply replaced by an amide function. This can be provided by an N-methylamide, an achiral or chiral amino acid ester, or a dipeptide ester fragment. Accordingly, the K-value raises to $K = 39$ in **2** ($R^1 = R^2$ = Ala-Ala-NH*Me*) and becomes even larger than 50 in **3** ($R^1 = R^2$ = Ala-Ala-Gly-O*Me*), **4** ($R^1 = R^2$ = Ala-Ala-Ala-O*Me*), and **5** ($R^1 = R^2$ = Ala-Ala-Ala-Ala-O*Me*). Thereby discriminating energies [11] may exceed 9.5 kJ mol^{-1}. The third amide linkage must serve as hydrogen bonding donor otherwise the influence of the peptidic chain becomes strongly lowered as revealed by the K-values of compounds **7** ($R^1 = R^2$ = Leu-Gly-Pro-O*Me*, $K = 9.0$) and **8** ($R^1 = R^2$ = Val-Val-Pro-O*Me*, $K = 1.4$) (Table 2). This decrease does not arise from mutual interferences of peptidic ligands as shown by the similar discrimination constants obtained for the biliverdin-XIII α bis(peptide) **27** ($R^1 = R^2$ = Leu-Gly-Pro-O*Me*, $K = 8.0$) and the corresponding monopeptide **30** (R^1 = Leu-Gly-Pro-O*Me*, R^2 = O*Me*; $K = 7.8$) (Table 3). On changing from chloroform to benzene – thus

minimizing intermolecular competition with the surrounding solvent – the helical geometry is preserved in bilipeptides of sequence Leu-Gly-Pro (**7**, **27**, and **30**) and the large discriminating forces are restored ($K > 50$). Compound **8** ($R^1 = R^2 =$ Val-Val-Pro-O*Me*) however behaves quite differently: In this case a deviation from the standard helical geometry must be assumed as indicated by the small *f*-value ($f = 2.11$).

In contradistinction to the third amide bond the donor properties of the second amino acid seem to be less important. Thus, if in **5** ($R^1 = R^2 =$ Ala-Ala-Ala-Ala-O*Me*) prolyl is substituted for the second alanyl unity affording **9** ($R^1 = R^2 =$ Ala-Pro-Ala-Ala-O*Me*), the helical conformation and the large discriminatory efficiency are preserved. The other bilipeptides of sequence X^1-Pro-X^3 **10–12** behave similar. The smaller *K*-value observed for **12** ($K = 23$) can be ascribed to the large hydrophobic groups of *all* three entities X^i and most probably comprises a steric influence preventing larger differences in intramolecular hydrogen bonding between *M* and *P* helices.

The importance of the donor properties of the first amino acid entity for helical standard conformation *and* large discriminatory efficiency becomes evident if bilipeptides of general sequence Pro-X^1-X^2 are considered. The peptide sequence Pro-Leu-Gly of **14** comprises a permutation of Leu-Gly-Pro as contained in **7**. However, while the conformation of **7** is close to that of the ester **6**, stretched conformers are present in **14**. This tendency becomes even more pronounced in **15**, **16**, and **17** and can be further increased on going from chloroform to apolar solvents like benzene. Characteristic for these conformational influences are the occasional interferences with a second peptide chain as present in biliverdin bis(peptides) arising in benzene solution. Thus, if the hydrophobic group of the amino acid entity *X* following proline is small as in **13** differences in conformation between mono- and bis-compounds occur. Accordingly, the *f*-value of the biliverdin-XIII α bis(peptide) *5* ($R^1 = R^2 =$ Pro-Ala-Ala-O*Me*) being similar to that of the ester **24** suggests a helical standard conformation but the corresponding monopeptide **28** ($R^1 =$ Pro-Ala-Ala-O*Me*, $R^2 =$ O*Me*) shows appreciable deviation (Table 3). If the hydrophobicity of X^1 becomes larger *both*, the mono- *and* bis-derivatives adopt a stretched conformation as revealed by the *f*-values of **26** and **29**. Other examples are contained in Ref. [5].

For bilipeptides of sequence Pro-X^1-X^2 the conformational constraints imposed on the bilatriene backbone to change the energetically most favourable (*syn, syn, syn*) conformation of (*Z, Z, Z*) bilatrienes has been ascribed to the formation of a γ-turn structure formed by hydrogen bonding between the propionic carbonyl and the second amide linkage. Due to the singularity of spatial orientations thus generated the remaining amide hydrogen of the C-terminal amino acid and one of the hydrogens located at the pyrrolinone nitrogens efficiently compete for hydrogen bonding with the pyrrolenine nitrogen. Thus, the corresponding intra-bilatriene hydrogen bond is weakened facilitating rotation around the C(5) methine single bond to form (*anti, syn, syn*) conformers (s. Fig. 1) [12]. Expectedly, similar changes in UV-VIS spectra occur if the (*Z, Z, Z, anti, syn, syn*) conformation is produced by insertion of an ethylene bridge between C(7) and N(21) as has been shown by Iturraspe et al. [13].

The co-operation of amino acid constituents (Pro, X^1, X^2) in stretching of the bilatriene moiety accounts for the ineffectiveness of the partial sequences Pro-X^1

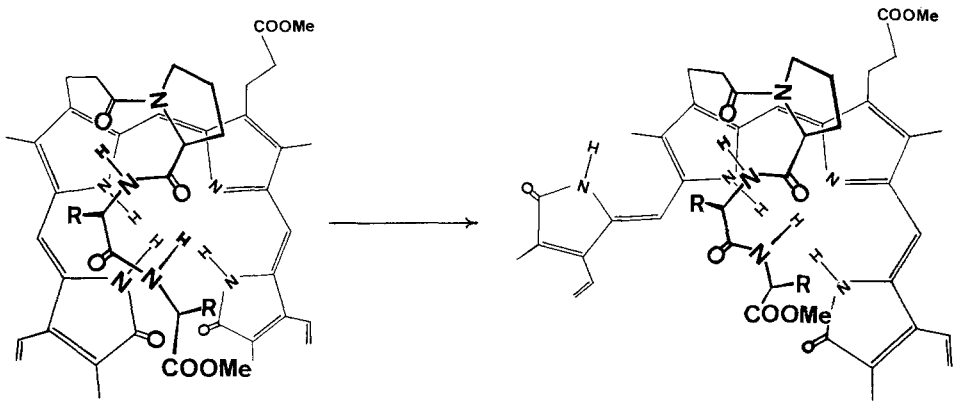


Fig. 1. Conformational transition from (*Z, Z, Z, syn, syn, syn*) to (*Z, Z, Z, anti, syn, syn*) in bilipeptides of sequence Pro-X^1-X^2. Note, that only one peptidic chain is needed. (In Ref. [5] the spatial arrangement of the propionic carbonyl involved in the γ-turn has been incorrectly reproduced)

and X^1-X^2: In **17** ($R^1 = R^2$ = Pro-Leu-Leu-O*Me*) appreciable amounts of *anti* conformers are present in chloroform and benzene. On the other hand the *f*- and *K*-values of **18** ($R^1 = R^2$ = Pro-Leu-O*Me*) and **19** ($R^1 = R^2$ = Leu-Leu-O*Me*) are indicative of the standard helical geometry along with poor discriminatory efficiency.

Stretching of the bilatriene moiety – albeit less pronounced – must also be suspected to be responsible for the lowered *f*-values of the biliverdin bis(pentapeptides) **21** and **22** if the solvent is changed from chloroform to benzene. This behaviour is reminescent of **8** ($R^1 = R^2$ = Val-Val-Pro-O*Me*) exhibiting deviation from the standard geometry only in benzene solutions.

For a decrease in *f*-values two kinds of conformational changes can be envisaged: (i) Increase of the helical pitch as a result of a small increase of torsional angles preserving the overall (*Z, Z, Z, syn, syn, syn*) geometry or (ii) formation of *anti* conformers by torsion around a methine single bond exceeding 90°. For bilipeptides of sequence Pro-X^1-X^2 (**14–17**, **26**, **28**, and **29**) the former possibility (i) has been ruled out [5] but for compounds **8**, **21**, and **22** both (i) and (ii) must be considered. On the other hand, the *mechanisms* accounting for stretching of the bilatriene chromophore must be different for these two sets of compounds. One distinguishing feature is the influence of the second peptidic chain in biliverdin bis(peptides). A comparison of *f*-value of the biliverdin bis(pentapeptide) **22** ($f = 1.81$) with that of the corresponding mono derivative **23** ($f = 2.28$) for solutions in benzene suggests that deviations from the standard helical bilatriene geometry arise from the co-operative action of *two* peptidic chains. No stretching tendency can be observed for **23**. This contrasts the behaviour of bilipeptides of sequence Pro-X^1-X^2 (vide supra).

Conclusions

To cause a pronounced conformational influence on the bilatriene backbone the peptidic entities in bilipeptides must contain at least three amide bonds. Thereby the hydrogen bonding donor and acceptor properties of the first N-terminal

and – although to a smaller extent – the third amide hydrogen regulate the conformation of the bilatriene moiety. Thus, complete transformation into (*Z, Z, Z, syn, syn, syn*) *P* helical bilatriene species takes place if both sites possess donor properties. Otherwise, if a prolyl unity void of hydrogen bonding donor ability provides one of these amide bonds, population of stretched conformers may become important.

$M \rightarrow P$ helical transition essentially arises from one single peptidic chain. A second, if present as with biliverdin bis(peptides), causes a decrease rather than an increase of discriminatory efficiency. Only if helical discrimination is low a priori and if the number of sites of the ligand capable of hydrogen bonding is small, as with biliverdin bis(amino acid esters) [2], co-operation of ligands may occur resulting in an enhancement of conformational influence.

For stretching [10] of the bilatriene backbone two mechanisms must be considered differing in the number of peptidic chains involved. The one becomes operative in bilipeptides of sequence Pro-X^1-X^2 in which the secondary structure of one single peptide ligand controls bilatriene conformation. Thereby interferences with a second chain, if present, may occur hindering the formation of the conformation determining secondary structure by intramolecular inter-chain interactions. By contrast, for the second mechanism, as operative in biliverdin bis(pentapeptides) of sequence X^1-X^2-Pro-X^3-X^4 the presence of two peptidic chains is prerequisite. This suggests formation of a distinct tertiary structure built up from adjacent peptide unities providing appropriate orientations of hydrogen bonding sites to those of the bilatriene moiety.

For the bilipeptides investigated so far the conformational influence of the peptide entities on the bilatriene moiety mainly arises from intramolecular hydrogen bonding and decreases with increasing intermolecular competition with the solvent. In ethanol the regulating influence of peptidic entities is mostly lost.

Experimental Part

Instruments and conditions for spectroscopic measurements were the same as used in the previous paper [5]. The peptide esters (as hydrochlorides) were prepared in a stepwise manner by the phosphorazo- and/or hydroxysuccinimide method starting from the C-terminal end. The diastereoisomeric homogeneity (94–98%) was assessed as described in Ref. [5]. Compounds **2**, **3**, **8**, **9**, **18**–**23**, **27**, and **30** were prepared according to the general procedures described in Ref. [5] (Yield 40–60%). For UV-VIS, CD, and ^{1}H NMR spectra see Tables 4, 5, and 6. Molar rotations of bilipeptides refer to ca. $2 \cdot 10^{-5}$ mol dm^{-3} solutions at 20 °C (error ± 5 000°).

Biliverdin-IXα bis(alanyl-alanine-N-methyl carboxamide) (**2**)

From biliverdin-IXα and (–) H-Ala-Ala-NH*Me* · HCl $\{[\alpha]_D^{20} -21.8°$ (*c* 3 in *Me*OH)$\}$; no m.p., gradually decomposing on heating up to 300 °C, *m/z* 893.0 ($M^+ + H$), $[M]_{546}^{20} -222\,000°$ (chloroform-methanol 98 : 2 *v/v*).

Biliverdin-IXα bis(alanyl-alanyl-glycine methyl ester) (**3**)

From biliverdin-IXα and (–) H-Ala-Ala-Gly-O*Me* · HCl $\{[\alpha]_D^{20} -32.6°$ (*c* 3 in *Me*OH)$\}$; no m.p., gradually decomposing on heating up to 300 °C, *m/z* 1 009.0 ($M^+ + H$), $[M]_{546}^{20} -223\,000°$ (chloroform-methanol 98 : 2 *v/v*).

Table 4. UV-VIS absorption spectra [ε_{max}/dm^3 mol^{-1} cm^{-1} (λ_{max}/nm)] of bilipeptides in various solvents of ca. $3 \cdot 10^{-5}$ mol dm^{-3} solutions at 20°C described in the present study

Compound	Benzene	Chloroform	Ethanol
2	[a]	13 700 (655)[b] 48 700 (377)[b]	[a]
3	[a]	14 100 (652)[b] 48 700 (378)[b]	[a]
8	16 800 (639) 48 400 (380)	14 500 (645) 53 600 (379)	14 500 (656) 51 800 (377)
9	13 300 (660) 47 600 (380)	12 600 (664) 45 100 (379)	13 400 (666) 48 000 (378)
18	15 200 (653) 48 100 (381)	13 500 (656) 50 300 (380)	13 300 (662) 49 500 (377)
19	14 800 (657) 48 100 (379)	13 600 (652) 51 900 (379)	13 600 (656) 50 200 (378)
20	[a]	14 200 (645) 44 500 (379)	13 700 (658) 47 700 (378)
21	15 200 (645) 38 800 (381)	12 900 (650) 45 600 (380)	12 600 (655) 46 200 (378)
22	18 900 (650) 39 500 (384)	14 800 (644) 44 100 (381)	12 500 (655) 46 500 (379)
23	15 100 (649) 45 500 (381)	13 200 (649) 49 000 (380)	13 000 (656) 48 500 (378)
27	15 300 (656) 37 800 (381)	14 600 (659) 38 900 (379)	14 500 (652) 39 600 (379)
30	15 300 (666) 37 300 (381)	14 400 (662) 36 800 (379)	14 000 (659) 37 800 (379)

[a] Insoluble in the solvent considered
[b] In chloroform-methanol (98 : 2 *v/v*)

Biliverdin-IXα bis(valyl-valyl-proline methyl ester) (**8**)

From biliverdin-IXα and (−) H-Val-Val-Pro-O*Me* · HCl {$[\alpha]_D^{20}$ −47.4° (*c* 2 in *Me*OH)}; m.p. 210–212 °C, *m/z* 1 201.5 ($M^+ + H$), $[M]_{546}^{20}$ −111 000° (benzene).

Biliverdin-IXα bis(alanyl-prolyl-alanyl-alanine methyl ester) (**9**)

From biliverdin-IXα and (−) H-Ala-Pro-Ala-Ala-O*Me* · HCl {$[\alpha]_D^{20}$ −136.5° (*c* 1 in *Me*OH)}; m.p. 215–218 °C, *m/z* 1 231.5 ($M^+ + H$), $[M]_{546}^{20}$ −232 000° (benzene).

Biliverdin-IXα bis(prolyl-leucine methyl ester) (**18**)

From biliverdin-IXα and (−) H-Pro-Leu-O*Me* · HCl {$[\alpha]_D^{20}$ −66.9° (*c* 2 in *Me*OH)}; m.p. 115–119 °C, *m/z* 1 031.0 ($M^+ + H$), $[M]_{546}^{20}$ −10 000° (benzene).

Table 5. CD spectra [$\Delta\varepsilon_{max}$/dm^3 mol^{-1} cm^{-1} (λ_{max}/nm)] of bilipeptides in various solvents of ca. $3 \cdot 10^{-5}$ mol dm^{-3} solutions at 20°C described in the present study

Compound	Benzene	Chloroform	Ethanol
2	[a]	+ 95.0 (655)[b] − 140.4 (376)[b]	[a]
3	[a]	+ 98.9 (655)[b] − 141.5 (377)[b]	[a]
8	+ 45.2 (635) − 72.3 (375)	+ 18.8 (645) − 31.3 (377)	+ 13.2 (645) − 24.0 (376)
9	+ 100.6 (658) − 141.5 (380)	+ 110.7 (657) − 153.1 (377)	+ 51.6 (660) − 68.6 (377)
18	+ 3.3 (658) − 6.9 (382)	+ 3.8 (660) − 9.6 (380)	+ 1.5 (660) − 5.3 (380)
19	+ 13.2 (650) − 20.4 (375)	+ 13.2 (650) − 22.0 (376)	+ 14.3 (660) − 24.0 (377)
20	[a]	+ 63.7 (650) − 102.1 (379)	+ 16.1 (650) − 26.9 (378)
21	+ 32.8 (635) − 46.6 (376)	− 8.6 (676) + 3.2 (583) + 11.6 (391)	+ 15.6 (650) − 23.8 (377)
22	+ 49.8 (642) − 71.3 (383)	+ 32.8 (640) − 47.9 (377)	+ 10.5 (645) − 16.1 (377)
23	+ 24.2 (655) − 36.7 (376)	+ 10.2 (650) − 14.6 (378)	+ 1.9 (650) − 2.9 (380)
27	+ 96.1 (655) − 115.6 (376)	+ 85.5 (655) − 106.7 (375)	+ 41.3 (660) − 52.7 (377)
30	+ 101.1 (658) − 120.1 (377)	+ 85.0 (658) − 103.2 (375)	+ 48.8 (658) − 60.7 (375)

[a] Insoluble in the solvent considered
[b] In chloroform-methanol (98 : 2 *v/v*)

Biliverdin-IXα bis(leucyl-leucine methyl ester) (**19**)

From biliverdin-IXα and (−) H-Leu-Leu-O*Me* · HCl {$[\alpha]_D^{20}$ −8.1° (*c* 2 in *Me*OH)}; m.p. 240–245 °C (decomp.), *m/z* 1063.5 (M^+ + H), $[M]_{546}^{20}$ −91 000° (benzene).

Biliverdin-IXα bis(alanyl-alanyl-prolyl-alanyl-alanine methyl ester) (**20**)

From biliverdin-IXα and (−) H-Ala-Ala-Pro-Ala-Ala-O*Me* · HCl {$[\alpha]_D^{20}$ − 132.6° (*c* 2 in *Me*OH)}; m.p. 223–226 °C (decomp.), *m/z* 1373.5 (M^+ + H), $[M]_{546}^{20}$ − 145 000° (chloroform).

Biliverdin-IXα bis(valyl-valyl-prolyl-alanyl-alanine methyl ester) (**21**)

From biliverdin-IXα and (−) H-Val-Val-Pro-Ala-Ala-O*Me* · HCl {$[\alpha]^{20}$ −106.9° (*c* 2 in *Me*OH)}; m.p. 162–167 °C, *m/z* 1486.0 (M^+ + H), $[M]_{546}^{20}$ −61 000° (benzene).

Table 6. ^{1}H NMR spectral data (δ/ppm) of bilipeptides **2**, **3**, **8**, **9**, **18–23**, **27**, and **30** at 250 MHz and 297 K[a]

Compound	Biliverdin moiety	Acyl substituents *R*
2[b]	6.96 (1 H, s, 10-H), 6.83 (1 H, m, 3-Vn-H_X), 6.56, 6.08, and 5.40 (1 H × 3, XMA, J_{XM} 17.0 Hz, J_{XA} 11.3 Hz, J_{MA} ca. 2 Hz, 18-Vn), 6.14 and 6.11 (1 H × 2, s, 5-H, 15-H), 5.72 (2 H, m, 3-Vn-H_{AB}), 2.83 (4 H, m, 8-CH_2, 12-CH_2), 2.35 (4 H, m, CH_2-CO), 2.18 (3 H, s, 17-*Me*), 2.10 and 2.05 (3 H × 2, s, 7-*Me*, 13-*Me*), 1.82 (3 H, s, 2-*Me*)	8.08 (2 H, d, *J* 7.3 Hz, N−H), 7.96 (2 H, d, *J* 7.3 Hz, N−H), 7.77 (2 H, q, *J* 4.4 Hz, NH-*Me*), 4.27 and 4.20 (2 H × 2, quintett, *J* 7.5 Hz, $C_\alpha-H$), 2.59 (6 H, d, *J* 4.4 Hz, N-*Me*), 1.20 and 1.14 (6 H × 2, d, *J* 7.5 Hz, Ala *Me*)
3[b]	6.94 (1 H, s, 10-H), 6.83 (1 H, m, 3-Vn-H_X), 6.56, 6.08, and 5.42 (1 H × 3, XMA, J_{XM} 17.5 Hz, J_{XA} 11.7 Hz, J_{MA} ca. 2 Hz, 18-Vn), 6.16 and 6.13 (1 H × 2, s, 5-H, 15-H), 5.70 (2 H, m, 3-Vn-H_{AB}), 2.83 (4 H, m, 8-CH_2, 12-CH_2), 2.35 (4 H, m, CH_2-CO), 2.18 (3 H, s, 17-*Me*), 2.10 and 2.06 (3 H × 2, s, 7-*Me*, 13-*Me*), 1.82 (3 H, s, 2-*Me*)	8.25 (2 H, t, *J* 5.8 Hz, Gly N−H), 8.06 and 8.03 (2 H × 2, d, *J* 7.5 Hz, Ala N−H), 4.29 and 4.30 (2 H × 2, quintett, *J* 7.5 Hz, Ala $C_\alpha-H$), c. 3.85 (4 H, m, Gly $C_\alpha-H$), 3.64 (6 H, s, COO*Me*), 1.21 and 1.13 (6 H × 2, d, *J* 7.5 Hz, Ala *Me*)
8[c, d]	6.98 (1 H, s, 10-H), 6.59 (1 H, m, 3-Vn-H_X), 6.49, 6.14, and 5.43 (1 H × 3, XMA, J_{XM} 17.5 Hz, J_{XA} 11.5 Hz, J_{MA} ca. 2 Hz, 18-Vn), 6.00 and 5.95 (1 H × 2, s, 5-H, 15-H), 5.64 (2 H, m, 3-Vn-H_{AB}), ca. 2.9 (4 H, m, 8-CH_2, 12-CH_2), ca. 2.5 (4 H, m, CH_2-CO), 2.15 (3 H, s, 17-*Me*), 2.06 and 2.04 (3 H × 2, s, 7-*Me*, 13-*Me*), 1.88 (3 H, s, 2-*Me*)	7.08 and 7.05 (1 H × 2, d, *J* ca. 7 Hz, N−H), 6.77 and 6.73 (1 H × 2, d, *J* ca. 8 Hz, N−H), 4.56, 4.40, and 4.30 (2 H × 3, m, $C_\alpha-H$), 3.82 and 3.60 (2 H × 2, m, Pro $C_\delta-H$), 3.65 (6 H, s, COO*Me*), 2.2–1.8 (12 H, m, Pro $C_\beta-$ and $C_\gamma-H$, Val $C_\beta-H$), 0.95, 0.88, 0.81, and 0.78 (6 H × 4, d, *J* ca. 7 Hz, Val *Me*)
9[c, d]	6.64 (1 H, m, 3-Vn-H_X), 6.53 (1 H, s, 10-H), 6.52, 6.15, and 5.46 (1 H × 3, XMA, J_{XM} 18.0 Hz, J_{XA} 11.0 Hz, J_{MA} 2.5 Hz, 18-Vn), 6.14 and 6.10 (1 H × 2, s, 5-H, 15-H), 5.80 (2 H, m, 3-Vn-H_{AB}), 3.2–2.5 (8 H, m, 8- and 12-CH_2-CH_2-CO), 2.21 (3 H, s, 17-*Me*), 2.10 and 2.07 (3 H × 2, s, 7-*Me*, 13-*Me*), 1.85 (3 H, s, 2-*Me*)	7.76 and 7.70 (1 H × 2, d, *J* 6.7 Hz, N−H), 6.76 and 6.73 (1 H × 2, d, *J* 7.0 Hz, N−H), 6.30 (1 H, d, *J* 7.5 Hz, N−H), ca. 6.15 (1 H, m, N−H), ca. 4.7 (4 H, m, Ala $C_\alpha-H$), ca. 4.4 (2 H, m, Pro $C_\alpha-H$), 4.18 (2 H, m, Ala $C_\alpha-H$), 3.69 (6 H, s, COO*Me*), 3.42 and 2.97 (2 H × 2, m, Pro $C_\delta-H$), 2.2–1.7 (8 H, m, Pro $C_\beta-$ and $C_\gamma-H$), 1.33 and 1.32 (3 H × 2, d, *J* 7.5 Hz, Ala *Me*), ca. 1.2 (12 H, m, Ala *Me*)
18[c, d]	6.78 (1 H, s, 10-H), 6.66 (1 H, m, 3-Vn-H_X), 6.45, 6.10, and 5.42 (1 H × 3, XMA, J_{XM} 17.4 Hz, J_{XA} 11.7 Hz, J_{MA} ca. 2 Hz, 18-Vn), 6.05 and 5.94 (1 H × 2, s, 5-H, 15-H), 5.63 (2 H, m, 3-Vn-H_{AB}), 2.92 (4 H, m, 8-CH_2, 12-CH_2), 2.52 (4 H, m, CH_2-CO), 2.15 (3 H, s, 17-*Me*), 2.07 and 2.06 (3 H × 2, s, 7-*Me*, 13-*Me*), 1.87 (3 H, s, 2-*Me*)	7.20 (2 H, d, *J* ca. 8 Hz, N−H), ca. 4.5 (4 H, m, $C_\alpha-H$), 3.70 (6 H, s, COO*Me*), 3.49 and 3.29 (2 H × 2, m, Pro $C_\delta-H$), 2.2–1.6 (14 H, m, Pro and Leu $C_\beta-$ and $C_\gamma-H$), ca. 0.9 (12 H, m, Leu *Me*)
19[c]	6.97 (1 H, s, 10-H), 6.58 (1 H, m, 3-Vn-H_X), 6.48, 6.14, and 5.43 (1 H × 3, XMA, J_{XM} 17.5 Hz, J_{XA} 11.0 Hz, J_{MA} ca. 2 Hz, 18-Vn), 5.99 and 5.93 (1 H × 2, s, 5-H, 15-H), 5.63 (2 H, m, 3-Vn-H_{AB}), 2.88 (4 H, m, 8-CH_2, 12-CH_2), 2.44 (4 H, m, CH_2-CO), 2.15 (3 H, s, 17-*Me*), 2.05 and 2.03 (3 H × 2, s, 7-*Me*, 13-*Me*), 1.87 (3 H, s, 2-*Me*)	7.08 and 7.04 (1 H × 2, d, *J* ca. 8 Hz, N−H), 6.95 and 6.90 (1 H × 2, d, *J* ca. 8 Hz, N−H), 4.43 (4 H, m, $C_\alpha-H$), 3.68 (6 H, s, COO*Me*), ca. 2.0 (4 H, m, Leu $C_\gamma-H$), 1.55 (8 H, m, Leu $C_\beta-H$), 0.85 (24 H, m, Leu *Me*)

Table 6 (continued)

Compound	Biliverdin moiety	Acyl substituents *R*
20[b, d]	6.91 (1 H, s, 10-H), 6.82 (1 H, m, 3-Vn-H_X), 6.56, 6.06, and 5.39 (1 H × 3, XMA, J_{XM} 17.0 Hz, J_{XA} 11.3 Hz, J_{MA} ca. 2 Hz, 18-Vn), 6.13 and 6.11 (1 H × 2, s, 5-H, 15-H), 5.71 (2 H, m, 3-Vn-H_{AB}), 2.81 (4 H, m, 8-CH_2, 12-CH_2), 2.32 (4 H, m, CH_2 − CO), 2.17 (3 H, s, 17-*Me*), 2.08 and 2.05 (3 H × 2, s, 7-*Me*, 13-*Me*), 1.80 (3 H, s, 2-*Me*)	8.16 (2 H, d, *J* 7.2 Hz, N − H), 8.07 (2 H, d, *J* 7.2 Hz, N − H), 7.99 (2 H, d, *J* 7.7 Hz, N − H), 6.93 (2 H, d, *J* 7.9 Hz, N − H), 4.43 (2 H, quintett, *J* ca. 7 Hz, Ala C_α − H), ca. 4.25 (8 H, m, C_α − H), 3.59 (6 H, s, COO*Me*), 3.5–3.2 (4 H, m, Pro C_δ − H), 2.2–1.8 (8 H, m, Pro C_β − and C_γ − H), 1.27, 1.20, 1.15, and 1.11 (6 H × 4, d, *J* ca. 7 Hz, Ala *Me*)
21[b, d]	6.95 (1 H, s, 10-H), 6.82 (1 H, m, 3-Vn-H_X), 6.56, 6.07, and 5.39 (1 H × 3, XMA, J_{XM} 17.8 Hz, J_{XA} 11.0 Hz, J_{MA} ca. 2 Hz, 18-Vn), 6.12 and 6.09 (1 H × 2, s, 5-H, 15-H), 5.70 (2 H, m, 3-Vn-H_{AB}), 2.82 (4 H, m, 8-CH_2, 12-CH_2), ca. 2.4 (4 H, m, CH_2 − CO), 2.17 (3 H, s, 17-*Me*), 2.08 and 2.04 (3 H × 2, s, 7-*Me*, 13-*Me*), 1.81 (3 H, s, 2-*Me*)	8.21 (2 H, d, *J* 7.3 Hz, N − H), 7.95 (4 H, m, N − H), 7.87 (2 H, d, *J* 8.5 Hz, N − H), ca. 4.25 (10 H, m, C_α − H), ca. 3.8 and 3.5 (2 H × 2, m, Pro C_δ − H), 3.59 (6 H, s, COO*Me*), 2.2–1.7 (12 H, m, Pro C_β − and C_γ − H, Val C_β − H), 1.27 and 1.19 (6 H × 2, d, *J* ca. 7 Hz, Ala *Me*), 0.89, 0.86, 0.75, and 0.72 (6 H × 4, m, Val *Me*)
22[b, d]	6.92 (1 H, s, 10-H), 6.83 (1 H, m, 3-Vn-H_X), 6.56, 6.07, and 5.38 (1 H × 3, XMA, J_{XM} 17.0 Hz, J_{XA} 11.7 Hz, J_{MA} ca. 2 Hz, 18-Vn), 6.13 and 6.10 (1 H × 2, s, 5-H, 15-H), 5.69 (2 H, m, 3-Vn-H_{AB}), 2.80 (4 H, m, 8-CH_2, 12-CH_2), 2.32 (4 H, m, CH_2 − CO), 2.16 (3 H, s, 17-*Me*), 2.07 and 2.04 (3 H × 2, s, 7-*Me*, 13-*Me*), 1.79 (3 H, s, 2-*Me*)	ca. 8.1 (6 H, m, N − H), 7.90 (2 H, d, *J* 8.0 Hz, N − H), 4.29 (8 H, m, C_α − H), 4.12 (2 H, m, C_α − H), 3.7–3.3 (4 H, m, Pro C_δ − H), 3.60 (6 H, s, COO*Me*), 2.2–1.7 (12 H, m, Pro C_β − and C_γ − H, Val C_β − H), 1.19 and 1.10 (6 H × 2, d, *J* 6.7 Hz, Ala *Me*), ca. 0.9 (24 H, m, Val *Me*)
23[b, e]	6.93 (1 H, s, 10-H), 6.82 (1 H, m, 3-Vn-H_X), 6.56, 6.06, and 5.39 (1 H × 3, XMA, J_{XM} 17.5 Hz, J_{XA} 11.5 Hz, J_{MA} ca. 2 Hz, 18-Vn), 6.13 and 6.10 (1 H × 2, s, 5-H, 15-H), 5.70 (2 H, m, 3-Vn-H_{AB}), 2.84 (4 H, m, 8-CH_2, 12-CH_2), 2.32 (4 H, m, CH_2 − CO), 2.16 (3 H, s, 17-*Me*), 2.07 and 2.05 (3 H × 2, s, 7-*Me*, 13-*Me*), 1.80 (3 H, s, 2-*Me*)	8.03 (3 H, m, N − H), 7.85 (1 H, d, *J* 7.0 Hz, N − H), 4.5–4.1 (5 H, m, C_α − H), 3.63 (6 H, s, COO*Me*), 3.5–3.2 (2 H, m, Pro C_δ − H), 2.2–1.7 (6 H, m, Pro C_β − and C_γ − H, Val C_β − H), ca. 1.15 (6 H, m, Ala *Me*), 0.87 (12 H, m, Val *Me*)
27[c, d]	6.68 (2 H, m, 3- and 17-Vn-H_X), 6.51 (1 H, s, 10-H), 6.13 (2 H, s, 5-H, 15-H), ca. 5.65 (4 H, m, 3- and 17-Vn-H_{AB}), 3.1–2.4 (8 H, m, 8- and 12-CH_2 − CH_2 − CO), 2.02 (6 H, s, 7-*Me*, 13-*Me*), 1.92 (6 H, s, 2-*Me*, 18-*Me*)	7.0–6.5 (4 H, m, N − H), ca. 4.45 (4 H, m, Pro and Leu C_α − H), ca. 4.1 and 3.7 (2 H × 2, m, Gly C_α − H), ca. 3.5 (4 H, m, Pro C_δ − H), 3.69 (6 H, s, COO*Me*), 2.3–1.4 (14 H, m, Pro and Leu C_β − and C_γ − H), ca. 0.85 (12 H, m, Leu *Me*)
30[c]	6.74 and 6.67 (1 H × 2, m, 3- and 17-Vn-H_X), 6.54 (1 H, s, 10-H), 6.24 and 6.08 (1 H × 2, s, 5-H, 15-H), 5.8–5.5 (4 H, m, 3- and 17-Vn-H_{AB}), 3.1–2.4 (8 H, m, 8- and 12-CH_2 − CH_2 − CO), 2.06, 1.96, 1.95, and 1.91 (3 H × 4, s, 2-*Me*, 7-*Me*, 13-*Me*, 18-*Me*)	6.67 (1 H, d, *J* 6.5 Hz, Leu N − H), 5.60, 4.26, and 3.66 (1 H × 3, XMA, $J_{XM} = J_{XA}$ 5.2 Hz, J_{MA} 18 Hz, Gly NH − CH_2), 4.63 (1 H, m, Leu C_α − H), 4.38 (1 H, m, Pro C_α − H), 3.73 and 3.70 (3 H × 2, s, COO*Me*), 3.65 and 3.30 (1 H × 2, m, Pro C_δ − H), 2.1–1.2 (7 H, m, Pro and Leu C_β − and C_γ − H), 0.88 (3 H, d, *J* 6.0 Hz, Leu *Me*), 0.78 (3 H, d, *J* 6.6 Hz, Leu *Me*)

[a] Assignments could simply be established by drawing a comparison with the corresponding biliverdin esters **6** and **24**, peptide esters, and N-benzyloxycarbonyl peptide esters

[b] *DMSO-d_6*

[c] $CDCl_3$

[d] Main rotamer (> 70%) *trans, trans* with respect to the acyl − proline amide bonds. The portion of *trans, cis* and *cis, cis* rotamers (< 30%) could not be determined with sufficient accuracy due to severe overlapping of resonance absorptions

[e] Main rotamer (> 90%) *trans* with respect to the acyl − proline amide bond

Biliverdin-IXα bis- and -mono-(alanyl-valyl-prolyl-alanyl-valine methyl ester) (**22** and **23**)

From biliverdin-IXα and (−) H-Ala-Val-Pro-Ala-Val-O*Me*·HCl $\{[\alpha]_D^{20} -100.0°$ (*c* 1 in *Me*OH)$\}$. Compound **22** had m.p. 157–159 °C, *m/z* 1 486.0 ($M^+ + H$), $[M]_{546}^{20} -100\,000°$ (benzene). Compound **23** had m.p. 135–140 °C, *m/z* 1 048.5 ($M^+ + H$), $[M]_{546}^{20} -53\,000°$ (benzene).

Biliverdin-XIIIα bis- and -mono-(leucyl-glycyl-proline methyl ester) (**27** and **30**)

From biliverdin-XIIIα and (−) H-Leu-Gly-Pro-O*Me*·HCl $\{[\alpha]_D^{20} -52.4°$ (*c* 1.3 in *Me*OH)$\}$. Compound **27** had m.p. 195–198 °C, *m/z* 1 145.5 ($M^+ + H$), $[M]_{546}^{20} -210\,000°$ (benzene). Compound **30** had m.p. 200–203 °C, *m/z* 878.5 ($M^+ + H$), $[M]_{546}^{20} -221\,000°$ (benzene).

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[8] These small fluctuations in *f*-values on changing the solvent can even be observed with biliverdins whose helical conformations are fixed [5]
[9] Differences in *f*-values between isomeric biliverdin-IXα and -XIIIα derivatives reflect the different distribution of vinyl groups rather than differences in geometry (Lehner H., Krois D., unpublished results)
[10] In this report the term *stretched species* is used to indicate an increase of the distance between pyrrolinone rings A and D, thus refering to both helical conformers by enlargement of the pitch, and *anti* conformers by rotation around methine single bonds
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