Peptide-mediated Chiral Discrimination in Bilipeptides: Biliverdin Di- and Tri-peptides

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Synthetic biliverdin di- and tri-peptides (**1**)—(**26**) have been investigated by c.d. and u.v.–visible absorption spectroscopy with regard to the influence of their peptide chains on chiral discrimination of the helical, optically labile bilatriene backbone. In general, chiral discrimination increases with the number of amide groups per sidechain; the excess of population in bilitripeptides may exceed 85%. The preferred helicity of the bilatriene moiety is primarily determined by the presence of chiral centres between amide groups while the extent of discrimination depends on the steric requirements of all amino acid constituents present. In biliverdin bis(peptides) intramolecular interchain interactions may occur, lowering discriminatory efficiencies. Chiral discrimination is monitored by c.d. and its origin discussed in terms of intramolecular amide–bilatriene hydrogen bonding.

Biliproteins are involved in both photosynthesis and photomorphogenesis.[1] Detailed knowledge about the influence of the protein entity on the conformationally flexible chromophore therefore comprises an important goal in biliprotein and, more generally, in chromoprotein research. In the initial stage of our systematic investigations we reported on the conformational influence on the chromophore exerted by covalently bound amino acids.[2,3] It was shown that the conformational changes induced only involve the helical nature of the rapidly interconverting[4] bilatriene helix giving rise to a distinct excess of population. No stretched conformers could be detected in solution. If both sidechains of biliverdin carry amino acid substituents they largely co-operate in chiral discrimination. Thus intramolecular interchain interaction is less important. In continuing our efforts in biliprotein research we now report our results on biliverdin di- and tri-peptides. To make interpretation easier this study is restricted to chromopeptides containing the building units alanine, valine, and glycine. Alanine and valine were used because of the quite different steric requirements of their hydrophobic residues at the chiral centre, glycine was used because of its intrinsic achirality.

Results and Discussion

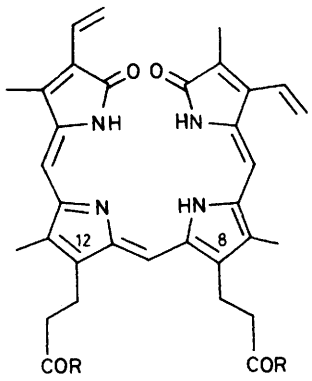
General.—In Tables 1 and 2 the c.d. and u.v.–visible spectral data of the biliverdin–peptides and –amino acid amides (**1**)—(**26**) are compiled. Both the visible band at *ca.* 660 nm and the u.v. band at *ca.* 380 nm belong to the bilatriene chromophore. They do not interfere with peptide transitions. To within 10 nm the c.d. and u.v.–visible maxima coincide. No spectral changes are observed within the concentration range 10^{-3}—10^{-6}M. Hence, as has been deduced for biliverdin amino acids chiral induction essentially occurs intramolecularly.[2,3] Conformational changes of the bilatriene backbone of compounds (**1**)—(**26**) other than inversion of the helix can largely be excluded. This is deduced from the great similarity of absorption spectra of compounds (**1**)—(**26**) in the same solvent. Moreover, the band positions and molar extinction coefficients are essentially the same as with biliverdin amino acid esters or even with achiral derivatives.[2,3] The coexistence of appreciable amounts of stretched conformers would have been reflected in the conformationally sensitive absorption spectra.[5] Except for compounds exhibiting small $\Delta\varepsilon$ values (*i.e.* long-wavelength c.d. band $\Delta\varepsilon \leqslant 10$) in chloroform as represented by the chromopeptides (**9**)—(**12**) and (**18**) chiral discrimination is markedly diminished for ethanol solutions. This follows from the magnitude of the pertinent c.d. parameters. The characteristics of these two groups have likewise been observed with biliverdin amino acid esters.[3]

Chiral Discrimination versus *Amino Acid Constituents and Sequence.*—From the c.d. data of the dipeptides (**2**)—(**5**) and (**11**) it can be concluded that it is the *N*-terminal amino acid which determines the helicity of the bilatriene moiety. Two examples are indicative: (i) the corresponding c.d. bands of the diastereoisomers (**3**) [R = (*S*)-Ala-(*S*)-Ala-OMe] and (**4**) [R = (*S*)-Ala-(*R*)-Ala-OMe] are equal in sign although the *C*-terminal alanine moieties are of antipodal chirality; (ii) the achiral glycine serving as *N*-terminal (**11**) but not as *C*-terminal amino acid (**2**) gives rise to a dramatic decrease of $\Delta\varepsilon$ values. Apparently, the influence of the *C*-terminal amino acid is restricted to the *extent* of chiral discrimination,† decreasing with increasing steric requirement. The same conclusions are arrived at if the dipeptides (**7**)—(**10**), unique in possessing (*S*)-valine as the *N*-terminal amino acid, and (**12**) are considered. However, a comparison of this set of compounds with the dipeptides (**2**)—(**5**) additionally indicates the steric influence of the *N*-terminal amino acid on chiral discrimination. By inspection of Table 1 it follows that chiral discrimination in biliverdin-IXα bis(dipeptides) possessing the same *C*-terminal amino acid is always significantly larger if the *N*-terminal amino acid is alanine rather than valine. Thus, both amino acid constituents in biliverdin dipeptides qualitatively exert the same steric influences on chiral discrimation. The interdependence between steric requirement and chiral discrimination is thus inverse to that observed for biliverdin amino acids.[2]

The most prominent feature of the biliverdin bis(tripeptides) (**13**)—(**17**) is the pronounced increase of optical activity compared with dipeptides. Again the less sterically crowded alanine turns out to be more effective than valine since the c.d. values of (**13**) [R = (*S*)-Ala-(*S*)-Ala-(*S*)-Ala-OMe] markedly exceed those of the homologue (**14**) [R = (*S*)-Val-(*S*)-Val-(*S*)-Val-OMe]. In contradistinction to dipeptides, however, an achiral glycine unit behaves quite differently. This follows from the c.d. spectra of the bis(tripeptides) (**15**)—(**17**), their respective amino acid sequence comprising the three possible permutations of two valine and one glycine units. The $\Delta\varepsilon$ values obtained for these isomers are generally large irrespective of the sequence. Surprisingly, their values are even larger than those of the

† Here and elsewhere the extent of chiral discrimination is associated with c.d. parameters in the absorption region of the chromophore, since the helical excess is reflected in the magnitude of the Cotton effects of the pertinent compounds.

Table 1. C.d. [$\Delta\varepsilon_{max.}$/l mol^{-1} cm^{-1} ($\lambda_{max.}$/nm)] and u.v.–visible electronic absorption spectra [$\varepsilon_{max.}$/l mol^{-1} cm^{-1} ($\lambda_{max.}$/nm)] of biliverdin-IXα bis(peptides) in chloroform and ethanol of *ca.* 10^{-4}M solutions at 20 °C[a]



Compound	Chloroform		Ethanol	
	C.d.	U.v.–visible	C.d.	U.v.–visible
(**1**) R = (*S*)-Ala-NHMe	+44.5 (665)	14 500 (657)	+24.2 (662)	13 670 (663)
	−61.9 (374)	50 900 (377)	−36.3 (377)	49 870 (378)
(**2**) R = (*S*)-Ala-Gly-OMe	+56.4 (657)	13 870 (655)	+30.9 (660)	14 880 (663)
	−90.7 (375)	53 170 (378)	−46.5 (376)	55 110 (377)
(**3**) R = (*S*)-Ala-(*S*)-Ala-OMe	+28.4 (655)	14 260 (652)	+9.5 (660)	14 140 (662)
	−43.9 (378)	53 510 (378)	−14.4 (375)	51 120 (377)
(**4**) R = (*S*)-Ala-(*R*)-Ala-OMe	+22.1 (658)	13 390 (655)	+13.8 (663)	14 520 (660)
	−36.6 (377)	50 210 (379)	−21.6 (376)	53 260 (377)
(**5**) R = (*S*)-Ala-(*S*)-Val-OMe	+16.2 (648)	13 840 (650)	+9.5 (656)	15 170 (663)
	−27.6 (377)	51 340 (378)	−16.6 (378)	53 940 (377)
(**6**) R = (*S*)-Val-NHMe	+41.3 (659)	13 320 (652)	+26.6 (662)	13 160 (658)
	−61.3 (376)	49 420 (378)	−39.4 (377)	48 230 (377)
(**7**) R = (*S*)-Val-Gly-OMe	+38.8 (659)	13 390 (655)	+22.2 (660)	13 470 (658)
	−62.8 (377)	48 290 (378)	−34.1 (375)	47 510 (377)
(**8**) R = (*S*)-Val-(*S*)-Ala-OMe	+20.2 (650)	13 770 (650)	+9.7 (650)	14 300 (660)
	−33.0 (378)	50 090 (378)	−14.5 (375)	49 700 (380)
(**9**) R = (*S*)-Val-(*S*)-Val-OMe	+9.6 (650)	14 910 (660)	+10.2 (650)	15 160 (663)
	−16.3 (377)	53 230 (379)	−15.2 (375)	51 810 (378)
(**10**) R = (*S*)-Val-(*R*)-Val-OMe	+7.2 (645)	14 660 (652)	+11.9 (655)	15 470 (660)
	−12.7 (377)	53 970 (379)	−19.3 (375)	55 190 (375)
(**11**) R = Gly-(*S*)-Ala-OMe	*b*	12 640 (657)	*ca.* −0.8 (*ca.* 680)	13 510 (665)
	ca. −0.8 (375)	48 390 (379)	+1.2 (383)	49 030 (376)
(**12**) R = Gly-(*S*)-Val-OMe	+2.2 (655)	12 140 (657)	−2.0 (670)	12 750 (665)
	−3.9 (378)	47 150 (378)	+2.6 (380)	46 980 (377)
(**13**) R = (*S*)-Ala-(*S*)-Ala-(*S*)-Ala-OMe	+109.0 (655)	13 460 (652)		
	−151.0 (375)[c,d]	45 950 (377)[c,e]	*c*	*c*
(**14**) R = (*S*)-Val-(*S*)-Val-(*S*)-Val-OMe	+72.0 (640)	14 350 (640)		
	−111.0 (378)[c,d]	45 740 (378)[c,e]	*c*	*c*
(**15**) R = (*S*)-Val-(*S*)-Val-Gly-OMe	+73.9 (640)	14 330 (640)		
	−112.2 (375)[f]	46 250 (377)[f]	*c*	*c*
(**16**) R = Gly-(*S*)-Val-(*S*)-Val-OMe	+80.2 (644)	14 550 (645)	+33.2 (660)	13 920 (658)
	−119.4 (376)	47 600 (377)	−37.2 (378)	43 380 (377)
(**17**) R = (*S*)-Val-Gly-(*S*)-Val-OMe	+97.3 (658)	13 670 (657)	+30.6 (662)	14 420 (665)
	−129.1 (378)	48 040 (378)	−40.4 (378)	48 870 (378)
(**18**) R = Gly-Gly-(*S*)-Val-OMe	+3.9 (650)	13 720 (657)	+8.2 (663)	14 630 (663)
	−4.1 (375)	48 040 (378)	−10.3 (379)	51 970 (378)

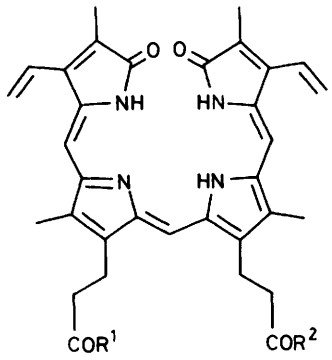
[a] $\lambda_{max.}$ ±10 nm (red band), ±2 nm (blue band). [b] $|\Delta\varepsilon|$ (600—700 nm) ≤0.8. [c] Insoluble in the solvent considered. [d] Extrapolated values from chloroform–methanol 2% v/v solutions; the c.d. for pure chloroform solutions is in general *ca.* 10% larger than for chloroform–methanol solutions; the values for this solvent are [$\Delta\varepsilon(\lambda)$]: (**13**) +99.5 (655), −137.6 (376); (**14**) +64.9 (641), −100.7 (378); (**15**) +63.8 (646), −98.0 (377); (**16**) +73.7 (647), −107.9 (377); (**17**) +87.4 (658), −115.1 (379). [e] Values refer to chloroform–methanol 2% v/v; in general, u.v.–visible spectra of biliverdin peptides do not substantially change if 2% v/v methanol is added. [f] Values refer to *ca.* 5 × 10^{-6}M solutions because of low solubility in chloroform.

homopeptide (**14**) consisting of three chiral amino acids. Only for the bis(tripeptide) (**18**) [R = Gly-Gly-(*S*)-Val-OMe], possessing two glycine units per sidechain, does chiral induction become very small.

Since the chiroptical properties of the enantiomerically pure bilatriene helix (long-wavelength band $\Delta\varepsilon$ 100—120) have been determined previously[6,7] the enantiomeric excess of bilatriene helices present in bili-bis(di-) and -(tri-peptides), each of which consists of two diastereoisomers, may now conveniently be computed. Hence, the strong c.d. spectra obtained for the tripeptides (**13**) and (**17**) [R = (*S*)-Val-Gly-(*S*)-Val-OMe] for chloroform solution indicate an excess of homochiral helices (h.e.) of at least 85%, corresponding to a discriminating free enthalpy[8] $\Delta G \geq 77$ kJ mol^{-1}.

Mechanism of Chiral Induction.—Biliverdin bis(dipeptides)

Table 2. C.d. [$\Delta\varepsilon_{max.}$/l mol^{-1} cm^{-1} ($\lambda_{max.}$/nm)] and u.v.–visible electronic absorption spectra [$\varepsilon_{max.}$/l mol^{-1} cm^{-1} ($\lambda_{max.}$/nm)] of biliverdin-XIIIα mono- and bis-(peptides) in chloroform and ethanol of *ca.* 10^{-4}M solutions at 20 °C[a]



Compound	Chloroform		Ethanol	
	C.d.	U.v.–visible	C.d.	U.v.–visible
(**19**) $R^1 = R^2$ = (*S*)-Val-NHMe	+36.9 (650)	14 050 (643)	+17.8 (658)	13 850 (653)
	−48.9 (373)	40 280 (376)	−29.3 (381)	39 200 (377)
(**20**) R^1 = (*S*)-Val-NHMe, R^2 = OMe	+63.5 (659)	12 540 (656)	+13.3 (658)	12 040 (655)
	−82.9 (373)	33 210 (377)	−17.2 (375)	34 880 (376)
(**21**) $R^1 = R^2$ = (*S*)-Ala-Gly-OMe	+55.4 (652)	14 780 (642)	+28.8 (655)	14 730 (653)
	−77.9 (372)	42 380 (376)	−36.3 (372)	43 820 (377)
(**22**) R^1 = (*S*)-Ala-Gly-OMe, R^2 = OMe	+55.0 (657)	14 410 (652)	+13.6 (657)	14 450 (655)
	−68.2 (373)	40 050 (376)	−16.3 (373)	41 800 (376)
(**23**) $R^1 = R^2$ = (*S*)-Val-(*S*)-Val-OMe	+10.8 (644)	14 028 (644)	+9.7 (646)	15 620 (650)
	−15.9 (380)	41 090 (380)	−14.1 (376)	44 680 (377)
(**24**) R^1 = (*S*)-Val-(*S*)-Val-OMe, R^2 = OMe	+9.5 (650)	14 060 (647)	+2.1 (640)	14 780 (655)
	−13.9 (373)	40 330 (377)	−3.8 (375)	42 370 (376)
(**25**) $R^1 = R^2$ = (*S*)-Val-Gly-(*S*)-Val-OMe	+79.2 (652)	13 450 (650)	+34.6 (660)	13 480 (653)
	−89.8 (375)	37 970 (376)	−44.9 (380)	38 860 (377)
(**26**) R^1 = (*S*)-Val-Gly-(*S*)-Val-OMe, R^2 = OMe	+96.6 (658)	13 900 (662)	+27.6 (662)	13 520 (658)
	−106.1 (377)	34 460 (378)	−30.7 (377)	38 040 (377)

[a] See footnote to Table 1.

provide three potential sites per sidechain, two amide bonds, and the ester carbonyl group, accessible for hydrogen bonding with the bilatriene moiety. Plausibly, interactions with the amide groups should be by far stronger than with the ester group. In consequence, only the chiral centre of the *N*-terminal amino acid situated between these co-ordination sites is involved, and therefore determines the predominant helicity of the bilatriene backbone. If this amino acid lacks chirality as in (**11**) and (**12**), c.d. spectra are extremely weak. On the other hand, the chirality centre, if any, of the *C*-terminal amino acid is situated outside of this 'chiral clamp'. On the basis of these considerations the *C*-terminal amino acid can only modify the extent of chiral discrimination by strengthening or weakening the hydrogen bonds formed between the amide groups and the bilatriene moiety. This is in accord with the c.d. spectra of compounds (**2**), (**3**), and (**5**) showing decreasing $\Delta\varepsilon$ values in the order $\Delta\varepsilon$(**2**) > $\Delta\varepsilon$(**3**) > $\Delta\varepsilon$(**5**). Similar relations are obtained if compounds (**7**)—(**9**) are considered. At least for compounds for which steric perturbation of the intramolecular hydrogen bonded arrangement may be regarded as small, as for the amino acid amides (**2**) and (**6**), this model further implies that discrimination for amides should be larger than for their corresponding methyl esters. Actually, this is true for the alanine *N*-methylamide (**1**) [$\Delta\varepsilon$(665) +44.5, chloroform] if the value of the methyl ester [$\Delta\varepsilon$(660) +32.0, chloroform][2] is taken into account. However, among the valine compounds [*N*-methylamide (**6**) $\Delta\varepsilon$(660) +41.3; methyl ester[2] $\Delta\varepsilon$(657) +45.2; both chloroform] this relationship is reversed. These findings strongly suggest that additional factors, depending on the amino acid constituents, must be taken into consideration in the peptide-mediated chiral discrimination of the bilatriene helix. Since the compounds under consideration [(**1**)—(**10**)] each carry two proximate sidechains intramolecular interchain interactions seem to be the most plausible explanation for the deviation observed. Clearly, this additional hydrogen bonding would compete with the bilatriene peptide interactions, causing a decrease of discriminatory forces. Since the relationship between h.e. and ΔG is approximately linear up to *ca.* 50% h.e. the $\Delta\varepsilon$ values of the bis(peptides) should be twice as large as those of mono(peptides) if in the former the proximate chains act independently. Even if for larger discriminations this linearity no longer holds true the values for bis(derivatives) in any case should be larger. That the two sidechains in biliverdin bis(dipeptides) interact with each other can be seen from the results obtained from biliverdin-XIIIIα-mono- and -bis-derivatives. As expected, the values for the bis(valine *N*-methylamide) of biliverdin-IXα (**6**) and -XIIIα (**19**) for chloroform solution are similar, but that of the mono-derivative (**20**) is considerably larger. For the related pairs of dipeptides (**21**)–(**22**) and (**23**)–(**24**), respectively, almost identical discriminations are found for the mono- and the corresponding bis-peptide. It is plausible that, owing to the additional competition of the protic solvent, interchain interactions become less efficient for ethanol solutions. Thus, for this protic solvent, chiral discrimination in the bis-derivatives (**19**), (**21**), and (**23**) always exceeds that of the corresponding mono-compounds (**20**), (**22**), and (**24**). Intramolecular interchain interactions are essentially absent in amino acid derivatives with hydrophobic groups bound to the chiral

centre. They may likewise occur if more polar amino acids are involved.[3] However, these attenuations are far less pronounced than with di- or tri-peptides (see below).

From the preceding discussion it becomes evident that the efficiency of interaction with the bilatriene moiety should increase with the number of sites in the sidechains capable of hydrogen bonding. Hence, the pronounced increase of chiral discrimination observed for the homotripeptides (**13**) and (**14**) compared with the corresponding dipeptides (**3**) and (**8**) may be attributed to the additional amide bond providing a third potent hydrogen bonding site per sidechain. This is corroborated by supplementary data obtained for the tripeptides (**15**)—(**18**). Apparently, since discriminations are large in (**15**)—(**17**), irrespective of the position of glycine, but rather small in (**18**), all three amide groups must be involved simultaneously. Similar to the conditions met in dipeptides the chiral centres situated between amide groups largely determine chiral discrimination. Thus, the respective amino acid units act as a whole, and the question of their relative contribution to chiral discrimination becomes essentially irrelevant. The significantly larger $\Delta\varepsilon$ values of compounds (**16**) and (**17**) compared with the homopeptide (**14**) are most likely due to a release of steric overcrowding. Typically, the c.d. spectra of (**14**) and (**15**) are very similar, consistent with the model that the chiral centre of the *C*-terminal amino acid is less important for chiral discrimination. The relation $\Delta\varepsilon(13) > \Delta\varepsilon(14)$ likewise reflects the sensitivity towards the steric requirements of the amino acid constituents. As with the bis(dipeptides) for chloroform solution appreciable interactions between the proximate sidechains take place, the $\Delta\varepsilon$ values of the mono(tripeptide) (**26**) being larger than those found for the bis(tripeptide) (**25**). On the other hand, ethanol causes these interferences to decrease.

We may conclude with the following remarks. A prerequisite for large chiral discriminations in bili-di- and -tri-peptides is the presence of chiral centres located between hydrogen bonding amide groups. The discriminating forces thus induced are weakened if the amino acids involved become more bulky. In essence, this holds true for bis(peptides) and mono(peptides), too. If more than one amide group per sidechain is present as in biliverdin bis(amino acid amides) or bis(peptides), intramolecular interchain interactions may occur lowering the discriminatory efficiency. Even if for some bilipeptides investigated here chromophore–peptide interactions can be regarded as large the helical conformation of the bilatriene moiety remains the most stable. This is important in view of the stretched conformations of the chromophore postulated for native biliproteins.[1] Whether more polar peptides in the sidechains, or the influence of the secondary structure, might give rise to such conformational changes remains to be investigated.

Experimental

General Directions.—M.p.s were determined with a Kofler–Reichert hot-stage apparatus. ^{1}H N.m.r. spectra (250 MHz; Fourier transform mode) were recorded with a Bruker WM 250 instrument at 20 °C for solutions in [$^2H_6$]DMSO, [$^2H_5$]-pyridine, CD_3OD, $CDCl_3$, and CF_3CO_2D using $SiMe_4$ as internal reference. Fast-atom bombardment mass spectrometry (f.a.b.m.s.) was performed with a Varian MAT 311A instrument equipped with spectrosystem 166 (butane-1,2,4-triol; Xe). U.v.–visible spectra were measured at 20 °C with a Perkin–Elmer Lambda 7 spectrometer (0.1—10 cm quartz cuvettes). The c.d. spectra were taken with a Jobin Yvon Mark III instrument carrying thermostatted (20 ± 1 °C) cylindrical quartz cuvettes (0.05—10 cm). As solvents for u.v.–visible and c.d. measurements, spectroscopic grade (Uvasol; Merck) chloroform (chromatographed on alumina prior to use), methanol, and ethanol were used. Optical rotations (10 cm path length) were obtained with a Perkin-Elmer 241 instrument for solutions in methanol, ethanol (Uvasol; Merck), and water.

All reactions were carried out under argon and protected from light. Tetrahydrofuran (THF) used for syntheses was distilled twice from lithium aluminium hydride. Water refers to distilled water. Column chromatography was performed on Kieselgel 60 (230—400 mesh; Merck) with chloroform (LiChrosolv; Merck) and methanol (p.A.; Merck) as eluants. For t.l.c. Kieselgel HF_{254} (Merck) was used. All solvents were flushed with argon prior to use.

Starting Materials.—Biliverdin-IXα and -XIIIα were obtained by literature procedures.[9] The dipeptides used for syntheses have been prepared *via* the phosphorazo[10] and hydroxysuccinimide[11] methods. For the syntheses of tripeptides only the hydroxysuccinimide method was employed. Amino acid *N*-methylamides were synthesized by the mixed anhydride method from the appropriate amino acid, isobutyl chloroformate, and methylamine.[12] The optical purity of the peptides (94—98%) was established by both ^{1}H n.m.r. and g.l.c.[13]

Biliverdin-IXα Peptides and Amides (**1**)—(**18**).—*General procedure.* To biliverdin-IXα (50 mg, 0.086 mmol) dissolved in THF (5 ml) and water (1 ml) were added successively under stirring and cooling (0 °C) the appropriate peptide or amino acid *N*-methylamide (as salt) (0.75 mmol), *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodi-imide hydrochloride (Merck) (150 mg, 0.75 mmol), and pyridine (60 μl, 0.75 mmol). After 24 h at room temperature chloroform (75 ml) and butan-2-ol (25 ml) were added. The organic layer was then washed twice with 0.001M-hydrochloric acid and subsequently with aqueous hydrogencarbonate and water. After evaporation of the solvents *in vacuo* the residue was chromatographed on silica gel (eluant chloroform–methanol 5% v/v). The main fraction was triturated with benzene–n-hexane and centrifuged. The supernatant was discarded and the remaining dark powder dried *in vacuo* (yields 40—60%).

Biliverdin-IXα *bis*-[(S)-*alanine* N-*methylamide*] (**1**). This compound was prepared from biliverdin-IXα and (+)-(*S*)-alanine *N*-methylamide hydroacetate[12] {$[\alpha]_D^{20}$ +13.7° (*c* 5 in H_2O)}; no m.p., gradually decomposing on heating up to 300 °C; *m/z* 751 (M^+ + 1); δ([$^2H_6$]DMSO) biliverdin moiety: 6.94 (1 H, s), 6.82 (1 H, m), 6.56 (1 H, m), 6.14 (1 H, s), 6.11 (1 H, s), 6.07 (1 H, m), 5.71 (2 H, m), 5.39 (1 H, m), 2.83 (4 H, m), 2.35 (4 H, m), 2.17 (3 H, s), 2.10 (3 H, s), 2.07 (3 H, s), and 1.82 (3 H, s); acyl substituents: 8.06 (2 H, d, *J* 7.3 Hz), 7.79 (2 H, q, *J* 4.3 Hz), 4.22 (2 H, quintet, *J* 7.3 Hz), 2.55 (6 H, d, *J* 4.3 Hz), and 1.14 (6 H, d, *J* 7.3 Hz).

Biliverdin-IXα *bis*-[(S)-*alanylglycine methyl ester*] (**2**). This compound was obtained from biliverdin-IXα and (+)-(*S*)-alanylglycine methyl ester hydrochloride[14] {$[\alpha]_D^{20}$ +10.6° (*c* 2 in MeOH)}; m.p. 218—220 °C; *m/z* 895 (M^+ + H); δ([$^2H_6$]-DMSO) biliverdin moiety: 6.93 (1 H, s), 6.83 (1 H, m), 6.57 (1 H, m), 6.14 (1 H, s), 6.12 (1 H, s), 6.07 (1 H, m), 5.69 (2 H, m), 5.40 (1 H, m), 2.83 (4 H, m), 2.35 (4 H, m), 2.18 (3 H, s), 2.09 (3 H, s), 2.06 (3 H, s), and 1.81 (3 H, s); acyl substituents: 8.26 (2 H, t, *J* 6.0 Hz), 8.09 (2 H, d, *J* 7.5 Hz), 4.33 (2 H, quintet, *J* 7.2 Hz), 3.82 (4 H, m), 3.62 (6 H, s), and 1.17 (6 H, d, *J* 7.2 Hz).

Biliverdin-IXα *bis*-[(S)-*alanyl*-(S)-*alanine methyl ester*] (**3**). This compound was obtained from biliverdin-IXα and (−)-(*S*)-alanyl-(*S*)-alanine methyl ester hydroformate[15] {$[\alpha]_D^{25}$ −33.0° (*c* 2 in EtOH)}; m.p. 225—228 °C (decomp.); *m/z* 895 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.93 (1 H, s), 6.83 (1 H, m), 6.56 (1 H, m), 6.14 (1 H, s), 6.11 (1 H, s), 6.07 (1 H, m), 5.70 (2 H, m), 5.40 (1 H, m), 2.81 (4 H, m), 2.33 (4 H, m), 2.17 (3 H, s), 2.08 (3 H, s), 2.05 (3 H, s), and 1.82 (3 H, s); acyl substituents: 8.32 (2 H, d, *J* 7.1 Hz), 8.05 (2 H, d, *J* 7.4 Hz), 4.33 (2 H, quintet, *J* 7.3

Hz), 4.24 (2 H, quintet, *J* 7.4 Hz), 3.61 (6 H, s), 1.26 (6 H, d, *J* 7.4 Hz), and 1.16 (6 H, d, *J* 7.1 Hz).

Biliverdin-IXα *bis*-[(S)-*alanyl*-(R)-*alanine methyl ester*] (**4**). This compound was obtained from biliverdin-IXα and (+)-(*S*)-alanyl-(*R*)-alanine methyl ester hydroformate[16] {$[\alpha]_D^{25}$ +40.2° (*c* 2 in EtOH)}; m.p. 210—212 °C; *m/z* 895 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.95 (1 H, s), 6.82 (1 H, m), 6.56 (1 H, m), 6.14 (1 H, s), 6.12 (1 H, s), 6.08 (1 H, m), 5.69 (2 H, m), 5.39 (1 H, m), 2.83 (4 H, m), 2.35 (4 H, m), 2.17 (3 H, s), 2.08 (3 H, s), 2.05 (3 H, s), and 1.80 (3 H, s); acyl substituents: 8.31 (2 H, d, *J* 7.2 Hz), 8.02 (2 H, d, *J* 7.2 Hz), 4.35 (2 H, quintet, *J* 7.2 Hz), 4.25 (2 H, quintet, *J* 7.2 Hz), 3.61 (6 H, s), 1.25 (6 H, d, *J* 7.2 Hz), and 1.15 (6 H, d, *J* 7.2 Hz).

Biliverdin-IXα *bis*-[(S)-*alanyl*-(S)-*valine methyl ester*] (**5**). This compound was obtained from biliverdin-IXα and (−)-(*S*)-alanyl-(*S*)-valine methyl ester hydrochloride[17] {$[\alpha]_D^{20}$ −17.7° (*c* 2.5 in MeOH)}; m.p. 214—218 °C; *m/z* 951 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.94 (1 H, s), 6.82 (1 H, m), 6.57 (1 H, m), 6.13 (1 H, s), 6.11 (1 H, s), 6.07 (1 H, m), 5.70 (2 H, m), 5.40 (1 H, m), 2.81 (4 H, m), 2.41 (4 H, m), 2.17 (3 H, s), 2.08 (3 H, s), 2.06 (3 H, s), and 1.82 (3 H, s); acyl substituents: 8.11 (2 H, d, *J* 7.2 Hz), 8.06 (2 H, d, *J* 7.3 Hz), 4.42 (2 H, quintet, *J* 7.2 Hz), 4.15 (2 H, t, *J* 7.2 Hz), 3.63 (6 H, s), *ca.* 2.0 (2 H, m), 1.16 (6 H, d, *J* 7.0 Hz), 0.89 (6 H, d, *J* 5.4 Hz), and 0.87 (6 H, d, *J* 5.4 Hz).

Biliverdin-IXα *bis*-[(S)-*valine* N-*methylamide*] (**6**). This compound was prepared from biliverdin-IXα and (+)-(*S*)-valine *N*-methylamide hydrochloride[12] {$[\alpha]_D^{20}$ +46.7° (*c* 1 in EtOH)}; no m.p., gradually decomposing on heating up to 300 °C; *m/z* 807 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.92 (1 H, s), 6.83 (1 H, m), 6.57 (1 H, m), 6.14 (1 H, s), 6.11 (1 H, s), 6.07 (1 H, m), 5.70 (2 H, m), 5.39 (1 H, m), 2.83 (4 H, m), 2.41 (4 H, m), 2.17 (3 H, s), 2.09 (3 H, s), 2.07 (3 H, s), and 1.82 (3 H, s); acyl substituents: 7.91 (2 H, q, *J* 4.6 Hz), 7.90 (2 H, d, *J* 8.1 Hz), 4.07 (2 H, t, *J* 8.1 Hz), 2.55 (6 H, d, *J* 4.6 Hz), *ca.* 1.9 (2 H, m), 0.75 (6 H, d, *J* 6.8 Hz), and 0.74 (6 H, d, *J* 6.8 Hz).

Biliverdin-IXα *bis*-[(S)-*valylglycine methyl ester*] (**7**). This compound was obtained from biliverdin-IXα and (+)-(*S*)-valylglycine methyl ester hydrochloride[18] {$[\alpha]_D^{20}$ +17.3° (*c* 2 in MeOH)}; no m.p., gradually decomposing on heating up to 300 °C; *m/z* 923 (M^+ + H); δ([$^2H_5$]pyridine) biliverdin moiety: 7.38 (1 H, s), 6.75 (2 H, m), 6.10 (1 H, s), 6.03 (1 H, s), 5.62 (4 H, m), 3.18 (4 H, m), 2.88 (4 H, m), 2.13 (3 H, s), 2.10 (3 H, s), 1.97 (3 H, s), and 1.94 (3 H, s); acyl substituents: 9.50 (2 H, t, *J* 4.0 Hz), 9.05 (2 H, d, *J* 7.7 Hz), 5.01 (2 H, m), 4.43 (2 H, m), 4.16 (2 H, m), 3.60 (6 H, s), 2.44 (2 H, m), 1.20 (6 H, d, *J* 7.0 Hz), and 1.16 (6 H, d, *J* 7.0 Hz).

Biliverdin-IXα *bis*-[(S)-*valyl*-(S)-*alanine methyl ester*] (**8**). This compound was obtained from biliverdin-IXα and (−)-(*S*)-valyl-(*S*)-alanine methyl ester hydrochloride[19] {$[\alpha]_D^{20}$ −7.6° (*c* 1 in EtOH)}; m.p. 259 °C (decomp.); *m/z* 951 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.94 (1 H, s), 6.83 (1 H, m), 6.58 (1 H, m), 6.13 (1 H, s), 6.11 (1 H, s), 6.08 (1 H, m), 5.70 (2 H, m), 5.39 (1 H, m), 2.81 (4 H, m), *ca.* 2.4 (4 H, m), 2.17 (3 H, s), 2.08 (3 H, s), 2.07 (3 H, s), and 1.82 (3 H, s); acyl substituents: 8.44 (2 H, d, *J* 5.2 Hz), 7.91 (2 H, d, *J* 8.2 Hz), 4.22 (4 H, m), 3.59 (6 H, s), *ca.* 1.9 (2 H, m), 1.27 (6 H, d, *J* 7.0 Hz), 0.83 (6 H, d, *J* 5.8 Hz), and 0.77 (6 H, d, *J* 6.0 Hz).

Biliverdin-IXα *bis*-[(S)-*valyl*-(S)-*valine methyl ester*] (**9**). This compound was obtained from biliverdin-IXα and (+)-(*S*)-valyl-(*S*)-valine methyl ester hydrochloride[20] {$[\alpha]_D^{20}$ +4.5° (*c* 4 in MeOH)}; m.p. 218—223 °C (decomp.); *m/z* 1 007 (M^+ + H); δ([$^2H_5$]pyridine) biliverdin moiety: 7.40 (1 H, s), 6.82 (1 H, m), 6.69 (1 H, m), 6.10 (1 H, s), 6.01 (1 H, s), 5.64 (4 H, m), 3.19 (4 H, m), 2.92 (2 H, m), 2.80 (2 H, m), 2.13 (3 H, s), 2.10 (3 H, s), 2.00 (3 H, s), and 1.97 (3 H, s); acyl substituents: 9.44 (2 H, d, *J ca.* 8 Hz), 9.11 (2 H, d, *J ca.* 8 Hz), 5.07 (2 H, t, *J ca.* 8 H), 4.89 (2 H, t, *J* 7.5 Hz), 3.70 (6 H, s), 2.39 (2 H, m), 2.29 (2 H, m), 1.14 (6 H, d, *J* 6.7 Hz), 1.10 (6 H, d, *J* 6.7 Hz), and 1.05 (12 H, d, *J* 6.5 Hz).

Biliverdin-IXα *bis*-[(S)-*valyl*-(R)-*valine methyl ester*] (**10**). This compound was obtained from biliverdin-IXα and (+)-(*S*)-valyl-(*R*)-valine methyl ester hydrochloride[21] {$[\alpha]_D^{20}$ +55.6° (*c* 2 in MeOH)}; m.p. 217—219 °C (decomp.); *m/z* 1 007 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.94 (1 H, s), 6.82 (1 H, m), 6.56 (1 H, m), 6.13 (1 H s), 6.10 (1 H, s), 6.06 (1 H, m), 5.70 (2 H, m), 5.39 (1 H, m), 2.83 (4 H, m), 2.45 (4 H, m), 2.17 (3 H, s), 2.09 (3 H, s), 2.06 (3 H, s), and 1.81 (3 H, s); acyl substituents: 8.27 (2 H, d, *J* 8.3 Hz), 7.87 (2 H, d, *J* 8.7 Hz), 4.40 (2 H, dd, J_1 8.7, J_2 6.5 Hz), 4.18 (2 H, dd, J_1 8.3, J_2 6.7 Hz), 3.63 (6 H, s), 2.04 (2 H, m), 1.90 (2 H, m), 0.86 (6 H, d, *J* 6.5 Hz), 0.85 (6 H, d, *J* 6.5 Hz), 0.80 (6 H, d, *J* 6.7 Hz), and 0.76 (6 H, d, *J* 6.7 Hz).

Biliverdin-IXα *bis*[*glycinyl*-(S)-*alanine methyl ester*] (**11**). This compound was obtained from biliverdin-IXα and (−)-glycinyl-(*S*)-alanine methyl ester hydrochloride[22] {$[\alpha]_D^{20}$ −49.1° (*c* 2 in MeOH)}; m.p. 187—189 °C (decomp.); *m/z* 867 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.95 (1 H, s), 6.83 (1 H, m), 6.56 (1 H, m), 6.14 (1 H, s), 6.10 (1 H, s), 6.07 (1 H, m), 5.71 (2 H, m), 5.40 (1 H, m), 2.83 (4 H, m), 2.37 (4 H, m), 2.17 (3 H, s), 2.08 (3 H, s), 2.06 (3 H, s), and 1.80 (3 H, s); acyl substituents: 8.30 (2 H, d, *J* 7.1 Hz), 8.11 (2 H, t, *J* 6.4 Hz), 4.28 (2 H, quintet, *J* 7.1 Hz), 3.70 (4 H, m), 3.60 (3 H, s), and 1.26 (6 H, d, *J* 7.1 Hz).

Biliverdin-IXα *bis*[*glycinyl*-(S)-*valine methyl ester*] (**12**). This compound was prepared from biliverdin-IXα and (−)-glycinyl-(*S*)-valine methyl ester hydrochloride[23] {$[\alpha]_D^{20}$ −16.6° (*c* 2 in MeOH)}; m.p. 162—164 °C; *m/z* 923 (M^+ + H); δ($CDCl_3$) biliverdin moiety: 7.10 (1 H, s), 6.60 (1 H, m), 6.47 (1 H, m), 6.12 (1 H, m), 6.00 (1 H, s), 5.94 (1 H, s), 5.63 (2 H, m), 5.42 (1 H, m), 2.96 (4 H, m), 2.56 (4 H, m), 2.14 (3 H, s), 2.07 (3 H, s), 2.05 (3 H, s), and 1.87 (3 H, s); acyl substituents: 7.63 (1 H, t, *J ca.* 5 Hz), 7.59 (1 H, t, *J ca.* 5 Hz), 7.40 (1 H, d, *J ca.* 8 Hz), 7.37 (1 H, d, *J ca.* 8 Hz), 4.45 (2 H, dd, J_1 8.1, J_2 5.4 Hz), 3.94 (4 H, m), 3.69 (6 H, s), *ca.* 2.2 (2 H, m), 0.94 (6 H, d, *J* 5.3 Hz), and 0.91 (6 H, d, *J* 5.3 Hz).

Biliverdin-IXα *bis*-[(S)-*alanyl*-(S)-*alanyl*-(S)-*alanine methyl ester*] (**13**). This compound was obtained from biliverdin-IXα and (−)-(*S*)-alanyl-(*S*)-alanyl-(*S*)-alanine methyl ester hydroacetate[24] {$[\alpha]_D^{20}$ −56.3° (*c* 0.15 in MeOH)}; no m.p., gradually decomposing on heating up to 300 °C; *m/z* 1 037 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.94 (1 H, s), 6.83 (1 H, m), 6.57 (1 H, m), 6.14 (1 H, s), 6.12 (1 H, s), 6.08 (1 H, m), 5.70 (2 H, m), 5.40 (1 H, m), 2.81 (4 H, m), 2.34 (4 H, m), 2.18 (3 H, s), 2.08 (3 H, s), 2.05 (3 H, s), and 1.81 (3 H, s); acyl substituents: 8.23 (2 H, d, *J* 6.8 Hz), 8.06 (2 H, d, *J* 7.3 Hz), 8.00 (2 H, d, *J* 7.3 Hz), 4.27 (6 H, m), 3.62 (6 H, s), 1.28 (6 H, d, *J* 7.3 Hz), 1.20 (6 H, d, *J* 7.3 Hz), and 1.13 (6 H, d, *J* 6.9 Hz).

Biliverdin-IXα *bis*-[(S)-*valyl*-(S)-*valyl*-(S)-*valine methyl ester*] (**14**). This compound was obtained from biliverdin-IXα and (−)-(*S*)-valyl-(*S*)-valyl-(*S*)-valine methyl ester hydrochloride[24,25] {$[\alpha]_D^{22}$ −48.5° (*c* 2 in H_2O)}; no m.p., gradually decomposing on heating up to 300 °C; *m/z* 1 205 (M^+ + H); δ($CDCl_3$–CD_3OD 10% v/v) biliverdin moiety: 6.93 (1 H, s), 6.66 (1 H, m), 6.54 (1 H, m), 6.16 (1 H, m), 6.13 (1 H, s), 6.08 (1 H, s), 5.71 (2 H, m), 5.47 (1 H, m), 2.95 (4 H, m), 2.65 (2 H, m), 2.50 (2 H, m), 2.22 (3 H, s), 2.14 (3 H, s), 2.10 (3 H, s), and 1.94 (3 H, s); acyl substituents: 4.38 (6 H, m), 3.73 (6 H, s), *ca.* 2.0 (6 H, m), and 0.84 (36 H, m).

Biliverdin-IXα *bis*-[(S)-*valyl*-(S)-*valylglycine methyl ester*] (**15**). This compound was obtained from biliverdin-IXα and (−)-(*S*)-valyl-(*S*)-valylglycine methyl ester hydrochloride {$[\alpha]_D^{20}$ −24.1° (*c* 2 in MeOH)}; no m.p., gradually decomposing on heating up to 300 °C; *m/z* 1 121 (M^+ + H); δ(CF_3CO_2D) biliverdin moiety: 7.76 (1 H, s), 6.69 (2 H, m), 6.62 (1 H, s), 6.50 (1 H, s), 6.41 (1 H, m), 5.91 (3 H, m), 3.37 (4 H, m), 2.92 (4 H, m), 2.35 (3 H, s), 2.34 (3 H, s), 2.32 (3 H, s), and 2.20 (3 H, s); acyl substituents: 4.55 (4 H, t, *J ca.* 7 Hz), 4.32 (4 H, s), 3.94 (6 H, s), *ca.* 2.1 (4 H, m), 1.09 (12 H, d, *J* 6.0 Hz), 0.96 (6 H, d, *J* 6.7 Hz), and 0.88 (6 H, d, *J* 6.7 Hz).

Biliverdin-IXα *bis*[*glycinyl*-(S)-*valyl*-(S)-*valine methyl ester*] (**16**). This compound was obtained from biliverdin-IXα and (−)-glycinyl-(*S*)-valyl-(*S*)-valine methyl ester hydrochloride {$[\alpha]_D^{20}$ −42.5° (*c* 2 in MeOH)}; m.p. 250 °C (decomp.); *m/z* 1 121 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.95 (1 H, s), 6.83 (1 H, m), 6.56 (1 H, m), 6.14 (1 H, s), 6.11 (1 H, s), 6.07 (1 H, m), 5.70 (2 H, m), 5.40 (1 H, m), 2.83 (4 H, m), 2.35 (4 H, m), 2.16 (3 H, s), 2.07 (3 H, s), 2.05 (3 H, s), and 1.81 (3 H, s); acyl substituents: 8.23 (2 H, d, *J* 7.6 Hz), 8.13 (2 H, t, *J* 5.6 Hz), 7.84 (2 H, d, *J* 8.7 Hz), 4.34 (2 H, dd, J_1 8.6, J_2 6.8 Hz), 4.11 (2 H, t, *J* 7.2 Hz), 3.75 (4 H, m), 3.60 (6 H, s), *ca.* 2.0 (4 H, m), and 0.85 (24 H, m).

Biliverdin-IXα *bis*-[(S)-*valylglycinyl*-(S)-*valine methyl ester*] (**17**). This compound was obtained from biliverdin-IXα and (+)-(*S*)-valylglycinyl-(*S*)-valine methyl ester hydrochloride {$[\alpha]_D^{20}$ +2.5° (*c* 2 in MeOH)}; m.p. 250—260 °C (decomp.); *m/z* 1 121 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.91 (1 H, s), 6.83 (1 H, m), 6.56 (1 H, m), 6.15 (1 H, s), 6.12 (1 H, s), 6.07 (1 H, m), 5.69 (2 H, m), 5.39 (1 H, m), 2.82 (4 H, m), 2.42 (4 H, m), 2.16 (3 H, s), 2.08 (3 H, s), 2.06 (3 H, s), and 1.82 (3 H, s); acyl substituents: 8.24 (2 H, t, *J* 5.2 Hz), 8.00 (2 H, d, *J* 8.5 Hz), 7.96 (2 H, d, *J* 8.5 Hz), 4.18 (2 H, t, *J ca.* 8 Hz), 4.12 (2 H, t, *J ca.* 8 Hz), 3.75 (4 H, m), 3.63 (6 H, s), *ca.* 1.9 (4 H, m), 0.87 (12 H, d, *J* 6.6 Hz), 0.81 (6 H, d, *J* 6.2 Hz), and 0.79 (6 H, d, *J* 6.2 Hz).

Biliverdin-IXα *bis*[*glycinylglycinyl*-(S)-*valine methyl ester*] (**18**). This compound was obtained from biliverdin-IXα and (−)-glycinylglycinyl-(*S*)-valine methyl ester hydrochloride {$[\alpha]_D^{20}$ −26.2° (*c* 2 in MeOH)}; m.p. 209 °C (decomp.); *m/z* 1 037 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.94 (1 H, s), 6.82 (1 H, m), 6.56 (1 H, m), 6.14 (1 H, s), 6.12 (1 H, s), 6.07 (1 H, m), 5.70 (2 H, m), 5.39 (1 H, m), 2.84 (4 H, m), 2.37 (4 H, m), 2.17 (3 H, s), 2.08 (3 H, s), 2.06 (3 H, s), and 1.82 (3 H, s); acyl substituents: 8.21 (2 H, t, *J* 5.1 Hz), 8.11 (2 H, d, *J* 7.5 Hz), 8.09 (2 H, t, *J* 5.5 Hz), 4.17 (2 H, t, *J* 7.5 Hz), 3.78 (4 H, d, *J* 5.2 Hz), 3.71 (4 H, d, *J* 5.5 Hz), 3.64 (6 H, s), *ca.* 2.0 (2 H, m), 0.87 (6 H, d, *J* 6.7 Hz), and 0.85 (6 H, d, *J* 6.7 Hz).

Biliverdin-XIIIα *Mono- and Bis-*(*peptides*) *and -*(*amides*).—*General procedure.* To biliverdin-XIIIα (50 mg, 0.086 mmol) dissolved in THF (5 ml) and water (1 ml) were added successively under stirring and cooling (0 °C) the appropriate peptide or the amino acid *N*-methylamide (as salt) (0.3 mmol), *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodi-imide hydrochloride (75 mg, 0.33 mmol), and pyridine (24 μl, 0.3 mmol). The reaction was quenched when the mono:bis ratio was *ca.* 1:1 (after 1—4 h; t.l.c., $R_{F(mono)} < R_{F(bis)}$). Work-up was done as described for the biliverdin-IXα derivatives (see above). Without further purification the mixture was then dissolved in MeOH (10 ml) and treated with 2,2-dimethoxypropane (1 ml) and toluene-*p*-sulphonic acid (25 mg). After 10 h at room temperature chloroform (50 ml) was added. The organic layer was washed with sodium hydrogencarbonate and water and then evaporated to dryness. Column chromatography was performed as described for the biliverdin-IXα–peptides (see above). The mono derivatives were eluted first followed by the bis derivatives. After trituration with a mixture of benzene–n-hexane and centrifugation the pure compounds were obtained (yields 40—60%). For optical rotations of (*S*)-valyl *N*-methylamide and the peptide esters used see the appropriate biliverdin-IXα-derivative.

Biliverdin-XIIIα *bis*-[(S)-*valine* N-*methylamide*] (**19**) *and biliverdin*-XIIIα *mono*-[(S)-*valine* N-*methylamide*] *monomethyl ester* (**20**). These compounds were obtained from biliverdin-XIIIα and (+)-(*S*)-valine *N*-methylamide hydrochloride.[12] Compound (**19**) had no m.p., gradually decomposing on heating up to 300 °C; *m/z* 807 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.88 (1 H, s), 6.82 (2 H, m), 6.10 (2 H, s), 5.70 (4 H, m), 2.80 (4 H, m), 2.39 (4 H, m), 2.05 (6 H, s), and 1.86 (6 H, s); acyl substituents: 7.88 (2 H, q, *J* 4.4 Hz), 7.86 (2 H, d, *J* 8.0 Hz), 4.06 (2 H, t, *J* 8.0 Hz), 2.55 (6 H, d, *J ca.* 4 Hz), *ca.* 2.2 (2 H, m), 0.77 (6 H, d, *J* 5.4 Hz), and 0.75 (6 H, d, *J* 5.4 Hz). Compound (**20**) had m.p. 250 °C (decomp.); *m/z* 709 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.89 (1 H, s), 6.81 (2 H, m), 6.09 (2 H, s), 5.69 (4 H, m), 2.83 (4 H, m), *ca.* 2.5 (2 H, m), 2.37 (2 H, m), 2.05 (6 H, s), and 1.85 (6 H, s); acyl substituents: 7.86 (1 H, d, *J* 6.8 Hz), 7.84 (1 H, q, *J* 3.8 Hz), 4.06 (1 H, t, *J* 6.8 Hz), 3.59 (3 H, s), 2.56 (3 H, d, *J* 3.8 Hz), *ca.* 2.1 (1 H, m), 0.77 (3 H, d, *J* 5.5 Hz), and 0.76 (3 H, d, *J* 5.5 Hz).

Biliverdin-XIIIα *bis*-[(S)-*alanylglycine methyl ester*] (**21**) *and biliverdin*-XIIIα *mono*-[(S)-*alanylglycine methyl ester*] *monomethyl ester* (**22**). These compounds were prepared from biliverdin-XIIIα and (+)-(*S*)-alanylglycine methyl ester hydrochloride.[14] Compound (**21**) had m.p. 255 °C (decomp.); *m/z* 867 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.89 (1 H, s), 6.81 (2 H, m), 6.10 (2 H, s), 5.70 (4 H, m), 2.81 (4 H, m), 2.34 (4 H, m), 2.05 (6 H, s), and 1.86 (6 H, s); acyl substituents: 8.25 (2 H, t, *J* 5.6 Hz), 8.08 (2 H, d, *J* 7.5 Hz), 4.31 (2 H, quintet, *J* 7.4 Hz), 3.81 (4 H, m), 3.60 (6 H, s), and 1.16 (6 H, d, *J* 7.2 Hz). Compound (**22**) had m.p. 227 °C (decomp.); *m/z* 739 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.89 (1 H, s), 6.81 (2 H, m), 6.10 (2 H, s), 5.69 (4 H, m), 2.86 (2 H, m), 2.81 (2 H, m), *ca.* 2.5 (2 H, m), 2.32 (2 H, m), 2.05 (6 H, s), and 1.85 (6 H, s); acyl substituents: 8.26 (1 H, t, *J* 5.3 Hz), 8.07 (1 H, d, *J* 7.4 Hz), 4.33 (1 H, quintet, *J* 7.4 Hz), 3.82 (2 H, m), 3.62 (3 H, s), 3.57 (3 H, s), and 1.16 (3 H, d, *J* 7.2 Hz).

Biliverdin-XIIIα *bis*-[(S)-*valyl*-(S)-*valine methyl ester*] (**23**) *and biliverdin*-XIIIα *mono*-[(S)-*valyl*-(S)-*valine methyl ester*] *monomethyl ester* (**24**). These compounds were prepared from biliverdin-XIIIα and (+)-(*S*)-valyl-(*S*)-valine methyl ester hydrochloride.[20] Compound (**23**) had m.p. 260 °C (decomp.); *m/z* 1 007 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.91 (1 H, s), 6.81 (2 H, m), 6.09 (2 H, s), 5.69 (4 H, m), 2.80 (4 H, m), *ca.* 2.4 (4 H, m), 2.04 (6 H, s), and 1.85 (6 H, s); acyl substituents: 8.17 (2 H, d, *J* 7.1 Hz), 7.91 (2 H, d, *J* 8.2 Hz), 4.32 (2 H, t, *J* 8.2 Hz), 4.10 (2 H, t, *J* 7.1 Hz), 3.60 (6 H, s), *ca.* 2.0 (4 H, m), 0.89 (6 H, d, *J* 6.7 Hz), 0.86 (6 H, d, *J* 6.7 Hz), 0.82 (6 H, d, *J* 6.7 Hz), and 0.77 (6 H, d, *J* 6.7 Hz). Compound (**24**) had m.p. 245 °C (decomp.); *m/z* 809 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.88 (1 H, s), 6.81 (2 H, m), 6.09 (1 H, s), 6.08 (1 H, s), 5.69 (4 H, m), 2.86 (2 H, m), 2.82 (2 H, m), 2.55 (2 H, m), 2.39 (2 H, m), 2.04 (6 H, s), and 1.85 (6 H, s); acyl substituents: 8.14 (1 H, d, *J* 7.8 Hz), 7.86 (1 H, d, *J* 8.4 Hz), 4.29 (1 H, t, *J* 8.2 Hz), 4.10 (1 H, t, *J* 7.8 Hz), 3.60 (3 H, s), 3.57 (3 H, s), *ca.* 2.0 (2 H, m), 0.90 (3 H, d, *J* 7.2 Hz), 0.87 (3 H, d, *J* 7.2 Hz), 0.83 (3 H, d, *J* 7.2 Hz), and 0.78 (3 H, d, *J* 7.2 Hz).

Biliverdin-XIIIα *bis*-[(S)-*valylglycinyl*-(S)-*valine methyl ester*] (**25**) *and biliverdin*-XIIIα *mono*-[(S)-*valylglycinyl*-(S)-*valine methyl ester*] *monomethyl ester* (**26**). These compounds were prepared from biliverdin-XIIIα and (+)-(*S*)-valylglycinyl-(*S*)-valine methyl ester hydrochloride. Compound (**25**) had m.p. 270 °C (decomp.); *m/z* 1 121 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.88 (1 H, s), 6.82 (2 H, m), 6.10 (2 H, s), 5.68 (4 H, m), 2.80 (4 H, m), 2.38 (4 H, m), 2.05 (6 H, s), and 1.86 (6 H, s); acyl substituents: 8.24 (2 H, t, *J ca.* 5 Hz), 8.00 (2 H, d, *J* 8.1 Hz), 7.96 (2 H, d, *J* 8.1 Hz), 4.17 (2 H, t, *J* 8.1 Hz), 4.11 (2 H, t, *J* 8.1 Hz), 3.76 (4 H, m), 3.63 (6 H, s), *ca.* 2.0 (4 H, m), 0.86 (6 H, d, *J* 6.7 Hz), 0.85 (6 H, d, *J* 6.7 Hz), 0.82 (6 H, d, *J* 6.7 Hz), and 0.80 (6 H, d, *J* 6.7 Hz). Compound (**26**) had m.p. 240 °C (decomp.); *m/z* 866 (M^+ + H); δ([$^2H_6$]DMSO) biliverdin moiety: 6.89 (1 H, s), 6.82 (2 H, m), 6.09 (2 H, s), 5.69 (4 H, m), 2.86 (2 H, m), 2.80 (2 H, m), *ca.* 2.6 (2 H, m), 2.38 (2 H, m), 2.06 (6 H, s), and 1.86 (6 H, s); acyl substituents: 8.24 (1 H, t, *J ca.* 5 Hz), 8.00 (1 H, d, *J* 7.8 Hz), 7.94 (1 H, d, *J* 7.8 Hz), 4.18 (1 H, t, *J* 7.8 Hz), 4.12 (1 H, t, *J* 7.8 Hz), 3.77 (2 H, m), 3.65 (3 H, s), 3.59 (3 H, s), *ca.* 2.0 (2 H, m), 0.87 (3 H, d, *J* 6.7 Hz), 0.86 (3 H, d, *J* 6.7 Hz), 0.82 (3 H, d, *J* 6.7 Hz), and 0.81 (3 H, d, *J* 6.7 Hz).

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