Monatshefte für Chemie 117, 1205–1217 (1986)

Monatshefte für Chemie
Chemical Monthly

Chiral Discrimination in Biliverdin (*S*)-Amino Acids, II [1]: Structural Variations of the Amino Acid Functional Groups**

Daniel Krois and **Harald Lehner***

Institut für Organische Chemie, Universität Wien, A-1090 Wien, Österreich

(Received 10 December 1985. Accepted 27 January 1986)

The Biliverdin-(*S*)-amino acid derivatives **2–21** have been synthesized, and are subject to a thorough c. d. and u. v.-vis. electronic absorption analysis in the bilatriene chromophoric region. It is shown that the extent of chiral discrimination of the bilatriene helices is particularly sensitive towards structural variations of the amino acids bound to the propionic side chains. Thus, a pronounced decrease of chiral induction occurs if hydrogen bonding between one of the two essential coordination sites of the amino acid entity and the bilatriene backbone is disturbed. Accordingly, derivatives of (*S*)-amino acid *t*-butyl esters (**3, 5, 7, 16,** and **17**) and *N*-substituted (*S*)-amino acids (**8–10, 20,** and **21**) generally display weak c. d. spectra. If additional polar groups are present in bis(amino acid) derivatives mutual interferences of the adjacent side chains must be taken into account. The attenuations of $\Delta\varepsilon$-values observed for the bis(serine) and bis(aspartic acid) compounds **14** and **15** thus are mainly due to intramolecular interchain interactions. The results provide evidence in support of the proposed mechanism of chiral discrimination in biliverdin amino acids.

(Keywords: Biliverdins; Chiral discrimination; Amino acids; C. d.; U.v.-vis)

Chirale Induktion in Biliverdin-(S)-Aminosäuren, II [1]: *Strukturelle Veränderungen der Aminosäurefragmente*

Die Biliverdin-(*S*)-aminosäurederivate **2**—**21** wurden dargestellt und ihre CD- und UV-VIS-Spektren im Absorptionsbereich des Bilatriens einer eingehenden Analyse unterworfen. Es zeigt sich, daß das Ausmaß der chiralen Induktion im Bilatrienteil gegenüber einer Strukturvariation der kovalent gebundenen Aminosäuren besonders empfindlich ist. So beobachtet man eine deutliche Verringerung des enantiomeren Überschusses der Bilatrienhelices, sobald die Wasserstoffbrückenbindungen zwischen einer der beiden maßgebenden Koordinationsstellen des Aminosäurefragments und dem Bilatrien gestört sind.

** Dedicated to Prof. Dr. *Kurt L. Komarek* on the occasion of his 60th birthday.

Dementsprechend zeigen Derivate von (*S*)-Aminosäure-*t*-butylestern (**3, 5, 7, 16** und **17**) sowie *N*-substituierten Aminosäuren (**8**—**10**, **20** und **21**) vergleichsweise schwache CD-Spektren. Für Bis(aminosäure)derivate muß eine gegenseitige Störung der beiden Seitenketten nur dann in Betracht gezogen werden, wenn zusätzliche polare Gruppen vorhanden sind. So sind die Schwächungen, die bei den Bis(serin)- und Bis(asparaginsäure)derivaten **14** und **15** beobachtet werden, im wesentlichen auf intramolekulare Wechselwirkungen der benachbarten Seitenketten zurückzuführen. Die erhaltenen Ergebnisse stützen den vorgeschlagenen Mechanismus für die chirale Induktion bei Biliverdin-Aminosäuren.

Introduction

Biliproteins serve as light receptors in both photosynthesis and photomorphogenesis. The interaction of their chromophoric groups, *i.e.* open chain tetrapyrrols, with the covalently bound apoproteins is of outstanding importance for the understanding of their function [2]. Therefore we recently [1, 3] have initiated a systematic study on that topic. Experimental evidence has been provided that the large chiral discriminations in biliverdin (*S*)-amino acid methyl esters are due to the presence of two coordination sites in the amino acid residue, the ester carbonyl group serving as hydrogen acceptor and the amide hydrogen serving as hydrogen donor. Induction occurs intramolecularily by non bonding interactions of the bilatriene backbone with its own side chains as long as neutral, non acidic media are considered [1, 3]. Meanwhile a similar study has appeared [4a]. In this connection it might be of general importance that the c. d. data reported for optically active derivatives of 2,3-dihydrobilatriene-abc-12-propionic acid likewise fit these conditions [4a, 5].

Since the steric requirements of the asymmetric carbon markedly influences chiral discrimination [1] additional information concerning the role of structural variations of the C- and N-terminal sites of covalently bound amino acids was of interest [6, 7]. This should provide further support to the mechanism of chiral induction in biliverdin amino acids. As in Part 1 of this series [1] the intensity of c. d. bands of the bilatriene moiety is used to monitor the enantiomeric excess of the kinetically labile helices.

Results and Discussion

Electronic Absorption and C.D. Spectra

The u.v.-vis. spectra of compounds **2**–**12** and **14**–**21** for benzene, chloroform, and ethanol solutions are compiled in Table 1. The two main bands of the bilatriene entities around $\lambda = 660$ nm and 380 nm show characteristics similar to those described previously [1]. Due to the

Table 1. *U.v.-vis. electronic absorption spectra of optically active derivatives of biliverdin-IXα* **2–12** *and* **14–21** *($c \sim 5 \cdot 10^{-5} M$) at 293 K in various solvents; ε, $M^{-1} cm^{-1}$ (λ, nm)*

	Benzene	Chloroform	Ethanol
2[a]	15510 (653) 51100 (380)	14610 (658) 53450 (378)	14460 (665) 51600 (377)
3	15050 (640) 47890 (380)	12310 (652) 47220 (379)	13390 (664) 49980 (377)
4[a]	14110 (653) 48090 (381)	14540 (658) 54750 (378)	13180 (665) 49900 (377)
5	14170 (650) 47460 (380)	12560 (655) 48160 (379)	12470 (662) 49730 (377)
6[a]	13640 (655) 47010 (379)	13170 (662) 47230 (377)	13470 (663) 48360 (376)
7	15060 (643) 49980 (381)	13270 (652) 51790 (378)	13870 (660) 51290 (377)
8	15230 (657) 50960 (381)	14660 (658) 54750 (379)	14290 (661) 52790 (377)
9	15070 (652) 47180 (382)	12840 (657) 46570 (382)	13670 (664) 49790 (378)
10	15090 (655) 46930 (381)	13350 (660) 47320 (381)	13790 (665) 48510 (377)
11	15450 (658) 54220 (380)	13950 (660) 55780 (378)	14630 (663) 54760 (377)
12	15860 (655) 52610 (382)	13370 (657) 53690 (380)	14110 (664) 55350 (377)
14	14700 (656)[b] 48740 (381)	12800 (656) 47000 (378)	14850 (662) 52630 (378)
15	14220 (653) 50240 (381)	12490 (658) 49610 (379)	13230 (659) 50150 (377)
16	14450 (650) 48940 (381)	12860 (654) 49200 (380)	13060 (663) 48290 (377)
17	14410 (655) 47690 (381)	13310 (657) 49530 (379)	13220 (663) 47910 (376)
18	14190 (655) 47740 (380)	13520 (660) 50430 (378)	13310 (664) 48750 (376)
19	14240 (655) 48360 (380)	13580 (658) 50350 (378)	13280 (665) 48040 (376)
20	15990 (657) 53100 (381)	13910 (657) 52010 (380)	14980 (663) 54810 (376)
21	15270 (654) 48630 (380)	14270 (653) 51600 (379)	13730 (661) 49770 (376)

[a] Values taken from Ref. [1].
[b] Due to the low solubility in benzene $c = 2 \cdot 10^{-6} M$.

Table 2. *C.d. data of optically active derivatives of biliverdin-IXα* **2–12** *and* **14–21** *($c \sim 5 \cdot 10^{-5} M$) at 293 K in various solvents; $\Delta\varepsilon$, $M^{-1} cm^{-1}$ (λ, nm); estimated error for $|\Delta\varepsilon| > 5$: ± 5%, for $|\Delta\varepsilon| < 5$: ± 10%, as not otherwise stated*

	Benzene	Chloroform	Ethanol
2[a]	+39.0 (657) −56.9 (380)	+31.6 (657) −47.4 (376)	+14.9 (663) −20.4 (377)
3	+12.2 (652) −19.8 (379)	+7.7 (650) −13.7 (376)	+6.5 (660) −10.4 (377)
4[a]	+39.9 (662) −64.3 (378)	+33.4 (660) −52.7 (377)	+21.4 (663) −31.7 (378)
5	+26.2 (661) −45.7 (380)	+11.2 (660) −21.1 (378)	+13.0 (660) −22.7 (379)
6[a]	+52.9 (660) −77.9 (378)	+45.2 (657) −67.8 (377)	+27.3 (663) −38.2 (377)
7	+19.8 (658) −33.1 (380)	+11.8 (650) −20.6 (378)	+13.5 (662) −22.8 (377)
8	+23.3 (660) −35.3 (380)	+7.3 (660) −11.3 (378)	+12.0 (661) −16.5 (377)
9	+3.1 (667) −5.5 (377)	[b] −0.6 (377)	+5.3 (659) −9.1 (378)
10	+3.8 (663) −6.0 (377)	[b] −1.4 (380)	+4.3 (660) −7.3 (377)
11	+52.0 (661) −78.9 (379)	+37.1 (660) −63.3 (377)	+7.4 (660) −12.9 (375)
12	+3.0 (663) −4.7 (∼400)	+1.6 (∼620) −5.1 (377)	−2.0 (∼685) +1.3 (∼400)
14	+28.8 (664)[c] −49.6 (378)	+24.1 (662) −42.4 (378)	+13.8 (660) −17.9 (377)
15	+29.9 (664) −49.1 (379)	+28.9 (660) −48.8 (376)	+9.5 (660) −13.9 (375)
16	+6.4 (656) −10.5 (377)	+2.0 (650) −3.9 (375)	+2.0 (660) −3.0 (375)
17	+6.9 (654) −10.9 (377)	+3.1 (650) −5.3 (377)	+1.6 (660) −2.7 (378)
18	+19.2 (663) −28.9 (377)	+17.2 (661) −26.1 (376)	+7.7 (660) −11.3 (375)
19	+22.0 (665) −34.6 (379)	+16.5 (662) −27.2 (377)	+7.8 (660) −11.4 (377)
20	+8.7 (662) −12.4 (380)	+2.2 (655) −3.4 (376)	+5.0 (660) −7.0 (376)
21	+8.0 (662) −10.7 (380)	+2.4 (660) −4.0 (378)	+4.7 (660) −6.9 (375)

[a] Values taken from Ref. [1].
[b] $|\Delta\varepsilon|$ (750–550) $\leqslant 0.5$.
[c] Due to the low solubility in benzene $c = 2 \cdot 10^{-6} M$.

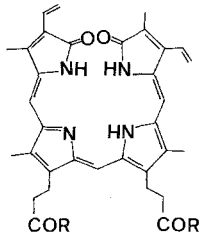
sensitivity of u.v.-vis. spectra towards conformational changes [8], it can be concluded that the helical conformation of the bilatriene backbone is not influenced by the amino acids covalently bound to the propionic side chains. The band positions of the c. d. spectra (Table 2) are very close to the respective u.v.-vis. spectra. Within experimental fluctuation the spectra gained are independent from concentration (10^{-6}–10^{-3} *M* solutions). This is in accordance with our previous findings that chiral discrimination of the bilatriene helices in biliverdin amino acids occurs intramolecularily [1].

For brevity, the following discussion is mostly devoted to benzene solutions because effects are most pronounced in this solvent but essentially the conclusions drawn are also valid for chloroform and ethanol solutions.

Discriminatory Efficiency vs. Structural Variations

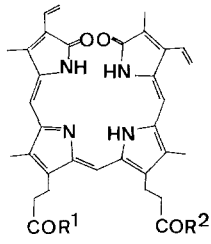
Considering first structure modifications at the C-terminal site of the amino acid moieties, the $\Delta\varepsilon$-values [9] measured for the *t*-butyl esters **3** ($\Delta\varepsilon = +12.2$), **5** ($\Delta\varepsilon = +26.2$), and **7** ($\Delta\varepsilon = +19.8$) are markedly decreased if compared with their corresponding methyl esters **2** ($\Delta\varepsilon = +39.0$), **4** ($\Delta\varepsilon = +39.9$), and **6** ($\Delta\varepsilon = +52.9$). Apparently, the reason for the attenuations of discriminatory forces is mainly steric, the *t*-butyl groups preventing as effective side chain—bilatriene interactions. Thus, steric influence of an alkyl group bound to the ester oxygen of the amino acid is opposite than for groups bound to the chirality centre [1].

Scheme 1



	R		*R*
1	OH	**9**	(*S*)-Pro-O*Me*
2	(*S*)-Ala-O*Me*	**10**	(*S*)-Pro-O*Bzl*
3	(*S*)-Ala-O*tBu*	**11**	(*S*)-Pro-Gly-O*Me*
4	(*S*)-Phe-O*Me*	**12**	(*S*)-Pro-Gly-O*tBu*
5	(*S*)-Phe-O*tBu*	**13**	(*S*)-Ser(*tBu*)-O*Me*
6	(*S*)-Val-O*Me*	**14**	(*S*)-Ser-O*Me*
7	(*S*)-Val-O*tBu*	**15**	(*S*)-Asp(O*Me*)-O*Me*
8	(*S*)-(*Me*)Val-O*Me*		

*Scheme 2**



	R^1	R^2
16	(*S*)-Ala-O*tBu*	O*Me*
17	O*Me*	(*S*)-Ala-O*tBu*
18	(*S*)-Asp(O*Me*)-O*Me*	O*Me*
19	O*Me*	(*S*)-Asp(O*Me*)-O*Me*
20	(*S*)-(*Me*)Val-O*Me*	O*Me*
21	O*Me*	(*S*)-(*Me*)Val-O*Me*

Structural variations at the N-terminal site of the amino acid entities is likewise accompanied by a modification of discriminatory interactions. This is reflected by the pronounced decrease of the $\Delta\varepsilon$-value obtained for the *N*-methyl valine methyl ester **8** ($\Delta\varepsilon = +23.3$) if compared with the unsubstituted compound **6** ($\Delta\varepsilon = +52.9$). Clearly, by *N*-methyl substitution the amino acid moiety, devoid of accessible hydrogen bonding donor groups, partly loses its interacting efficiency. From these results chiral discrimination in the proline esters **9** and **10** can be expected to be poor. This expectations conform with the weak c. d. spectra of compounds **9** ($\Delta\varepsilon = +3.1$) and **10** ($\Delta\varepsilon = +3.8$). The reason for the even smaller values may be interpreted in terms of rotational restrictions not present in the open chain amino acid derivative **8**. The optimal spacial accomodation of the potential coordinating binding sites upon the bilatriene entity might thus be further hindered. In the prolylglycine methyl ester **11** ($\Delta\varepsilon = +52.0$) the conditions for the generation of large discriminatory forces are met again: the peptidic bond connecting the (*S*)-proline and glycine building blocks now serves as hydrogen donor, while the second coordination site is provided by the ester carbonyl group of the glycine entity. Thus, even though glycine is intrinsically achiral the interactions with the bilatriene moiety receive their handedness from the (*S*)-proline unit inbetween. Moreover, as opposed to the proline esters **9** and **10** rotational restrictions no longer concern the binding sites. The involve-

* The assignment of isomers is arbitrarily.

ment of the ester carbonyl group of glycine is supported by the exceptionally small $\Delta\varepsilon$-value obtained for the *t*-butyl ester **12** ($\Delta\varepsilon = +3.0$).

One might argue that the influence of structure on chiral discrimination observed in compounds **3**, **5**, and **7**–**10** is due to intramolecular interchain interactions. Since the side chains in positions 8 and 12 both carry amino acids a mutual influence cannot be excluded, even if the independence of the side chains has been documented for undisturbed biliverdin bis(amino acid methyl esters) [1]. The c. d. measurements performed with the monoamino acid derivatives **16** ($\Delta\varepsilon = +6.4$) and **17** ($\Delta\varepsilon = +6.9$) clearly show that the contributions of the individual side chains in the bis(*t*-butyl ester) **3** ($\Delta\varepsilon = +12.2$) are fairly additive [10]. Likewise, the $\Delta\varepsilon$-value of the bis(*N*-methyl valine) **8** ($\Delta\varepsilon = +23.3$) roughly compares with the sum of the two isomeric monoderivatives **20** ($\Delta\varepsilon = +8.7$) and **21** ($\Delta\varepsilon = +8.0$). For chloroform and ethanol solutions similar relations can be taken from Table 2. Only the $\Delta\varepsilon$-value of compound **3** ($\Delta\varepsilon = +6.5$) for ethanol solution more strongly deviates from additivity (**16**: $\Delta\varepsilon = +2.0$ and **17**: $\Delta\varepsilon = +1.6$). Anyhow, if the interferences of the two side chains are held responsible for this discrepancy, this would indicate that the adjacent side chains mutually *increase,* rather than decrease, their discriminating effects. Hence, neither the attenuations observed for the *t*-butyl esters **3**, **5**, and **7** nor the weak c. d. spectra obtained for compounds lacking the amide hydrogen (**8**, **9**, and **10**) are due to intramolecular interchain interactions.

Now, the question of the role of additional polar groups in the amino acid moieties arises. Such groups are present in the (*S*)-serine and (*S*)-aspartic acid derivatives, **14** (hydroxymethyl) and **15** (carboxymethyl), respectively. Since steric influences of these groups can plausibly be excluded, the c. d. spectra of **14** ($\Delta\varepsilon = +28.8$) and **15** ($\Delta\varepsilon = +29.9$) may be compared with the value of the (*S*)-alanine derivative **2** ($\Delta\varepsilon = +39.0$). Obviously, both the hydroxymethyl groups of the bis(serine) derivative **14** and the additional estercarbonyl groups of the bis(aspartic acid) compound **15** cause a reduction of the enantiomeric excess of the bilatriene helices. However, as can be seen from the c. d. spectra of the monoaspartic acid derivatives **18** ($\Delta\varepsilon = +19.2$) and **19** ($\Delta\varepsilon = +22.0$) the individual contribution of each of the two side chains to chiral discrimination in the bisderivative **15** ($\Delta\varepsilon = +29.9$) is pronouncedly smaller. On the other hand, the sum [10] of $\Delta\varepsilon$-values of the monoaspartic acid compounds **18** and **19** ($= +41.2$), is very similar to the $\Delta\varepsilon$-value obtained for the bis(alanine) **2** ($\Delta\varepsilon = +39.0$). This indicates that additional polar groups do not compete with the other coordination sites, the amide group and the ester carbonyl group directly bound to the chirality centre of the pertinent amino acid, since this would reduce the $\Delta\varepsilon$-values of both the bis- *and*

mono-derivatives. More plausibly, it is the additional polar groups in the amino acid moiety of the bis(amino acid) derivative **15** which give rise to appreciable interactions between the two side chains. In consequence, these interferences concomitantly loosen discriminating forces. The c. d. of the bis(serine) derivative **14** may be explained by similar arguments. The reason for these attenuations is in contradistinction to that deduced for the *t*-butyl esters **3**, **5**, and **7** and the *N*-substituted amino acid derivatives **8**–**10**, respectively.

The results obtained are compatible with our recent postulate [1] that for the generation of large discriminating forces both the N- and C-terminal sites of the amino acid entities must effectively interact with the bilatriene moiety. If the structural features of the amino acid hinder this highly efficient mechanism, discriminations are markedly lowered.

By a closer inspection of the c. d. spectra (Table 2) two groups of compounds may be distinguished. The characteristics of the first group is that their $\Delta\varepsilon$-values pronouncedly decrease with increasing polarity of the solvent, the attenuations for ethanol solutions being largest. This class comprises the methyl esters **2**, **4**, and **6**, the dipeptide **11**, and the derivatives of the polar amino acids **14**, **15**, **18**, and **19**. Further examples are provided by Refs. [1] and [11]. For the second group this solvent dependence is partly lost. If compared with the $\Delta\varepsilon$-values for chloroform solutions the corresponding values for ethanol are very similar or, surprisingly, even *increased*. This is true for compounds **3**, **5**, **7**–**10**, **12**, **16**, **17**, **20**, and **21**, in which discriminatory forces *a priori* have been strongly reduced by structural variations. In marked contrast, no simple relationship between c. d. and a property of the solvent (e.g. dielectric constant, dipole moment) is evident. Similar irregularities have been observed with bili di- and tripeptides of small discriminatory efficiency [11].

Acknowledgements

We are grateful to Miss *D. Pawelczak* and Miss *S. Reiter* for skillful help with the experimental work. Thanks are due to Dr. *W. Silhan* and Dr. *A. Nikiforov* (from this institute) for recording n.m.r. and mass spectra, respectively. Financial support by the *Hochschuljubiläumsstiftung der Stadt Wien* and by the *Fonds zur Förderung der wissenschaftlichen Forschung* (under projects 3033, 4009, P 3574, and P 5767) is gratefully acknowledged.

Experimental

M.p.s. were taken with a *Kofler*-Reichert hot-stage apparatus. ^{1}H N.m.r. spectra (250 MHz, *Fourier* transform mode) were measured with a Bruker WM 250 instrument at 20 °C in deuteriochloroform (chromatographed on aluminia prior to use) and dimethylsulfoxide-d_6. As internal standard tetra-

methylsilane was used. Mass spectra were taken with a Varian MAT 311 A instrument equipped with spectrosystem 166 using the fast atom bombardement (f. a. b.) technique (butane-1,2,4-triol; Xe). U.v.-vis. spectra were taken with a Perkin-Elmer Lambda 7 instrument (0.1–10 cm quartz celles) at 20 °C. The c. d. spectra were recorded with a Jobin Yvon Mark III instrument carrying thermostated (20 ± 1 °C) cylindrical quartz cuvettes (0.1–10 cm). As solvents for u.v.-vis. and c. d. measurements spectroscopic grade (Uvasol; Merck) benzene, chloroform, and ethanol were used. Chloroform was chromatographed on aluminia prior to use. Optical rotations were obtained with a Perkin-Elmer 241 instrument (10 cm cuvettes).

All reactions were carried out under argon and protected from light. Column chromatography was performed on Kieselgel 60 (230–400 mesh; Merck) with chloroform (Lichrosolv, Merck), ethyl acetate (p.A., Fluka), and methanol (p.A., Merck) as eluants. Preparative t.l.c. was performed on Kieselgel 60 (Merck) using chloroform-methanol (1% *v/v*) as eluant. All solvents were flushed with argon.

The preparation of biliverdin-IXα (**1**) has been described elsewhere [12]. The isomeric biliverdin-IXα monomethyl esters were synthesized from **1** (100 mg) by partial esterification with 2,2-dimethoxypropane (1 ml) in methanol (10 ml) and catalytic amounts of methanolic hydrochloric acid at 20 °C. After 2 h the reaction mixture was quenched with water and extracted with chloroform—2-butanol (25% *v/v*). Unreacted **1** (20 mg) was stripped with aqueous sodium hydrogen carbonate. The organic layer was evaporated off *in vacuo* yielding 50 mg (61%) of a mixture (ca. 1 : 1) of biliverdin-IXα monomethyl esters.

Coupling of biliverdins with amino acid derivatives and dipeptides

Compounds **3**, **5**, **7–13**, and **15** were prepared following the general procedure described in Ref. [1]. Starting from **1** (50 mg, 0.086 mmol), the appropriate optically pure (*S*)-amino acid derivative (0.75 mmol), and *N*-ethyl-*N'*(3-dimethylaminopropyl)carbodiimide hydrochloride (Sigma). For the synthesis of the monoamino acid derivatives **16–21** the isomeric mixture of biliverdin-IXα monomethyl esters (*vide supra*) was used instead of **1**. The dipeptides **11** and **12** were prepared according to a modified procedure given in Ref. [11]. After column chromatography on silica gel [eluant for **3**, **5**, **7–10**, **13**, and **15**: ethyl acetate—chloroform (25% *v/v*); eluant for **11** and **12**: chloroform—methanol (3% *v/v*)] and trituration with benzene—hexane followed by centrifugation the pure compounds (yields 30–50% unless stated otherwise) were obtained.

Separation of the isomeric monoamino acid derivatives (**16/17**, **18/19**, **20/21**) was accomplished by preparative t.l.c. The syntheses procede without racemisation as has been shown in Ref. [1].

The c. d. and u.v.-vis. data are compiled in Tables 1 and 2.

Biliverdin-IXα bis[(S)-alanine t-butyl ester] (**3**)

From **1** and (*S*)-alanine *t*-butyl ester hydrochloride [13] {$[\alpha]_{546}^{20} = +1.8°$ ($c = 2$, ethanol)}, m.p. 163–165 °C, m.s.: 837 ($M + 1$); ^{1}H n.m.r. ($CDCl_3$, δ): Biliverdin moiety: 6.91 (1 H, s), 6.63 (1 H, m), 6.50 (1 H, m), 6.15 (1 H, m), 6.05 (1 H, s), 5.98 (1 H, s), 5.67 (2 H, m), 5.45 (1 H, m), 2.97 (4 H, m), 2.44 (4 H, m), 2.16 (3 H, s), 2.10 (3 H, s), 2.08 (3 H, s), 1.91 (3 H, s); acyl substituent: 6.73, 4.42, and 1.29 (5 H, AMX_3, $J_{AM} = 8.1$ Hz, $J_{MX} = 7.2$ Hz), 6.70, 4.42, and 1.29 (5 H, AMX_3, $J_{AM} = 8.1$ Hz, $J_{MX} = 7.2$ Hz), 1.44 (18 H, s).

Biliverdin-IXα bis[(S)-phenylalanine t-butyl ester] (**5**)

From **1** and (*S*)-phenylalanine *t*-butylester hydrochloride [13] {$[\alpha]_D^{25} = +46.6°$ ($c = 2$, ethanol)}, m.p. 137–140 °C, m.s.: 989 ($M+1$), ^{1}H n.m.r. ($CDCl_3$, δ): Biliverdin moiety: 6.93 (1 H, s), 6.63 (1 H, m), 6.51 (1 H, m), 6.15 (1 H, m), 6.02 (1 H, s), 5.96 (1 H, s), 5.67 (2 H, m), 5.45 (1 H, m), 2.95 (4 H, m), 2.43 (4 H, m), 2.18 (3 H, s), 2.08 (3 H, s), 2.06 (3 H, s), 1.90 (3 H, s); acyl substituent: 7.19 (6 H, m), 7.05 (4 H, m), 6.67 (1 H, d, $J \sim 8$ Hz), 6.64 (1 H, d, $J \sim 8$ Hz), 4.71 (2 H, q, $J = 6.8$ Hz), 2.98 (4 H, d, $J = 6.3$ Hz), 1.37 (18 H, s).

Biliverdin-IXα bis[(S)-valine t-butyl ester] (**7**)

From **1** and (*S*)-valine *t*-butyl ester hydrochloride [13] {$[\alpha]_D^{25} = +21.3°$ ($c = 2$, ethanol)}, m.p. 122–124 °C, m.s.: 893 ($M+1$), ^{1}H n.m.r. ($CDCl_3$, δ): Biliverdin moiety: 6.91 (1 H, s), 6.63 (1 H, m), 6.52 (1 H, m), 6.17 (1 H, m), 6.05 (1 H, s), 5.99 (1 H, s), 5.68 (2 H, m), 5.47 (1 H, m), 2.98 (4 H, m), 2.48 (4 H, m), 2.19 (3 H, s), 2.12 (3 H, s), 2.09 (3 H, s), 1.91 (3 H, s); acyl substituent: 6.59 (1 H, d, $J \sim 8$ Hz), 6.56 (1 H, d, $J \sim 8$ Hz), 4.38 (2 H, dd, $J_1 = 8.2$ Hz, $J_2 = 4.7$ Hz), ~2.0 (2 H, m), 1.42 (18 H, s), 0.84 (12 H, d, $J = 6.5$ Hz).

Biliverdin-IXα bis[(S)-N(methyl)valine methyl ester] (**8**)

From **1** and (*S*)-*N*(methyl)valine methyl ester hydrochloride [14] {$[\alpha]_D^{20} = +27.8°$ ($c = 2$, methanol)}, yield: 10%, m.p. 80–82 °C, m.s.: 837 ($M+1$), ^{1}H n.m.r. ($CDCl_3$, δ) [15]: Biliverdin moiety: 6.88 (0.25 H, s), 6.87 (0.25 H, s), 6.86 (0.5 H, s), 6.65 (1 H, m), 6.53 (1 H, m), 6.15 (1 H, m), 6.10 (1 H, s), 6.05 (1 H, s), 5.67 (2 H, m), 5.45 (1 H, m), 2.98 (4 H, m), 2.60 (4 H, m), 2.20 (3 H, s), 2.13 (3 H, s), 2.11 (3 H, s), 1.91 (3 H, s); acyl substituent: 4.98 (0.5 H, d, $J = 10.5$ Hz), 4.97 (1 H, d, $J = 10.5$ Hz), 3.90 (0.5 H, d, $J = 10.5$ Hz), 3.70 (1.5 H, s), 3.68 (1.5 H, s), 3.67 (3 H, s), 2.94 (4.5 H, s), 2.88 (1,5 H, s), ~2.1 (2 H, m), 0.98 (4.5 H, d, $J = 6.3$ Hz), 0.93 (1.5 H, d, $J = 6.3$ Hz), 0.86 (1.5 H, d, $J = 6.3$ Hz), 0.84 (4.5 H, d, $J = 6.3$ Hz).

Biliverdin-IXα bis[(S)-proline methyl ester] (**9**)

From **1** and (*S*)-proline methyl ester hydrochloride [13] {$[\alpha]_D^{24} = -31.8°$ ($c = 2$, methanol)}, m.p. 119–124 °C, m.s.: 805 ($M+1$), ^{1}H n.m.r. ($CDCl_3$, δ): Biliverdin moiety: 6.86 (1 H, s), 6.65 (1 H, m), 6.53 (1 H, m), 6.15 (1 H, m), 6.11 (1 H, s), 6.06 (1 H, s), 5.69 (2 H, m), 5.47 (1 H, m), 2.98 (4 H, m), 2.56 (4 H, m), 2.21 (3 H, s), 2.12 (3 H, s), 2.10 (3 H, s), 1.95 (3 H, s); acyl substituent: 4.48 (2 H, m), 3.73 (6 H, s), 3.59 (2 H, m), 3.44 (2 H, m), ~2.0 (8 H, m).

Biliverdin-IXα bis[(S)-proline benzyl ester] (**10**)

From **1** and (*S*)-proline benzyl ester hydrochloride [13] {$[\alpha]_D^{28} = -42.3°$ ($c = 1$, methanol)}, m.p. 97–100 °C, m.s.: 957 ($M+1$), ^{1}H n.m.r. ($CDCl_3$, δ): Biliverdin moiety: 6.85 (1 H, s), 6.65 (1 H, m), 6.53 (1 H, m), 6.15 (1 H, m), 6.11 (1 H, s), 6.05 (1 H, s), 5.68 (2 H, m), 5.46 (1 H, m), 2.97 (4 H, m), 2.55 (4 H, m), 2.20 (3 H, s), 2.12 (3 H, s), 2.10 (3 H, s), 1.92 (3 H, s); acyl substituent: 7.30 (10 H, s), 5.20 and 5.12 (4 H, AB, $J_{AB} = 12.7$ Hz), 4.55 (2 H, m), 3.57 (2 H, m), 3.44 (2 H, m), ~2.0 (8 H, m).

Biliverdin-IXα bis[(S)-prolylglycine methyl ester] (**11**)

From **1** and (*S*)-prolylglycine methyl ester hydrochloride [16] $\{[\alpha]_D^{29} = -41.2°$ ($c = 2$, methanol)}, m.p. 177–179 °C (dec.), m.s.: 919 ($M+1$), ^{1}H n.m.r. (*DMSO-d_6*, δ) [15]: Biliverdin moiety: 6.95 (1 H, s), 6.84 (1 H, m), 6.56 (1 H, m), 6.15 (0.4 H, s), 6.13 (0.6 H, s), 6.12 (0.4 H, s), 6.11 (0.6 H, s), 6.07 (1 H, m), 5.70 (2 H, m), 5.40 (1 H, m), 2.82 (4 H, m), ~2.5 (4 H, m), 2.17 (3 H, s), 2.10 (1.8 H, s), 2.08 (1.8 H, s), 2.07 (1.2 H, s), 2.05 (1.2 H, s), 1.82 (3 H, s); acyl substituent: 8.45 (0.8 H, m), 8.22 (1.2 H, m), 4.31 (2 H, m), 3.80 (4 H, m), 3.63 (3.6 H, s), 3.55 (2.4 H, s), ~3.5 (4 H, m), ~1.9 (8 H, m).

Biliverdin-IXα bis[(S)-prolylglycine t-butyl ester] (**12**)

From **1** and (*S*)-prolylglycine *t*-butyl ester $\{[\alpha]_D^{25} = -53.3°$ ($c = 2$, ethanol)}, m.p. 173–176 °C (dec.), m.s.: 1005 ($M+1$), ^{1}H n.m.r. (*DMSO-d_6*, δ) [15]: Biliverdin moiety: 6.95 (1 H, s), 6.83 (1 H, m), 6.56 (1 H, m), 6.16 (0.4 H, s), 6.14 (0.6 H, s), 6.13 (0.4 H, s), 6.10 (0.6 H, s), 6.07 (1 H, m), 5.70 (2 H, m), 5.40 (1 H, m), 2.82 (4 H, m), ~2.5 (4 H, m), 2.17 (3 H, s), 2.10 (1.8 H, s), 2.08 (1.8 H, s), 2.07 (1.2 H, s), 2.04 (1.2 H, s), 1.81 (3 H, s); acyl substituent: 8.32 (0.8 H, m), 8.11 (1.2 H, m), 4.30 (2 H, m), 3.67 (4 H, m), ~3.5 (4 H, m), ~1.9 (8 H, m), 1.40 (10.8 H, s), 1.35 (7.2 H, s).

Biliverdin-IXα bis[(S)-serine(tBu) methyl ester] (**13**)

From **1** and (*S*)-serine(*tBu*) methyl ester hydrochloride (Serva) $\{[\alpha]_D^{20} = +8.6°$ ($c = 2$, methanol)}, m.p. 87–92 °C, m.s.: 897 ($M+1$), ^{1}H n.m.r. ($CDCl_3$, δ): Biliverdin moiety: 6.88 (1 H, s), 6.64 (1 H, m), 6.52 (1 H, m), 6.16 (1 H, m), 6.07 (1 H, s), 6.01 (1 H, s), 5.68 (2 H, m), 5.47 (1 H, m), 2.98 (4 H, m), 2.48 (4 H, m), 2.19 (3 H, s), 2.12 (3 H, s), 2.10 (3 H, s), 1.91 (3 H, s); acyl substituent: 6.75 (1 H, d, $J = 8.3$ Hz), 6.70 (1 H, d, $J = 8.3$ Hz), 4.70 (2 H, m), 3.74 (2 H, m), 3.68 (3 H, s), 3.67 (3 H, s), 3.49 (2 H, m), 1.09 (9 H, s), 1.08 (9 H, s).

Biliverdin-IXα bis[(S)-serine methyl ester] (**14**)

A solution of 10 mg **13** (0.011 mmol) in 0.3 ml trifluoroacetic acid was allowed to stand at room temperature for 30 min. Then, chloroform was added and the organic layer extracted with aqueous sodium hydrogen carbonate. After evaporation of the solvent under reduced pressure the residue was chromatographed on silica gel with chloroform—methanol (3% *v/v*) as eluent to afford 5 mg (57%) **14**, m.p. 168–170 °C (dec.), m.s.: 785 ($M+1$), ^{1}H n.m.r. (*DMSO-d_6*, δ): Biliverdin moiety: 6.94 (1 H, s), 6.84 (1 H, m), 6.57 (1 H, m), 6.15 (1 H, s), 6.13 (1 H, s), 6.08 (1 H, m), 5.71 (2 H, m), 5.40 (1 H, m), 2.83 (4 H, m), 2.38 (4 H, m), 2.17 (3 H, s), 2.08 (3 H, s), 2.05 (3 H, s), 1.81 (3 H, s); acyl substituent: 8.23 (2 H, d, $J = 7.6$ Hz), 5.04 (2 H, broad), 4.36 (2 H, m), 3.60 (6 H, s), ~2.5 (4 H, m).

Biliverdin-IXα bis[(S)-aspartic acid dimethyl ester] (**15**)

From **1** and (*S*)-aspartic acid dimethyl ester hydrochloride [17] $\{[\alpha]_D^{20} = +15.8°$ ($c = 2$, methanol)}; m.p. 141–142 °C, m.s.: 869 ($M+1$), ^{1}H n.m.r. ($CDCl_3$, δ): Biliverdin moiety: 6.77 (1 H, s), 6.62 (1 H, m), 6.49 (1 H, m), 6.16 (1 H, m), 6.01 (1 H, s), 5.94 (1 H, s), 5.66 (2 H, m), 5.46 (1 H, m), 2.95 (4 H, m), 2.48 (4 H, m), 2.17 (3 H, s), 2.10 (3 H, s), 2.08 (3 H, s), 1.90 (3 H, s); acyl substituent: 6.95, 4.80, 2.90, and 2.73 (8 H, AMXY, $J_{AM} = 8.1$ Hz, $J_{MX} = J_{MY} = 4.8$ Hz, $J_{XY} = 16.7$ Hz), 3.70 (6 H, s), 3.65 (6 H, s).

Biliverdin-IXα mono[(S)-alanine t-butyl ester]monomethyl ester: Isomers **16** *and* **17**

From the isomeric mixture of biliverdin-IXα monomethyl esters and (*S*)-alanine *t*-butyl ester; R_f(**16**) > R_f(**17**). **16**: M.p. 146–150 °C, m.s.: 724 ($M+1$), ^{1}H n.m.r. ($CDCl_3$, δ): Biliverdin moiety: 6.87 (1 H, s), 6.64 (1 H, m), 6.52 (1 H, m), 6.16 (1 H, m), 6.09 (1 H, s), 6.03 (1 H, s), 5.68 (2 H, m), 5.47 (1 H, m), 2.96 (4 H, m), 2.60 (2 H, m), 2.45 (2 H, m), 2.19 (3 H, s), 2.12 (3 H, s), 2.09 (3 H, s), 1.91 (3 H, s); acyl substituent: 6.36, 4.47, and 1.31 (5 H, AMX_3, J_{AM} = 7.5 Hz, J_{MX} = 7.2 Hz), 3.67 (3 H, s), 1.45 (9 H, s).

17: M.p. 154–156 °C, m.s.: 724 ($M+1$), ^{1}H n.m.r. ($CDCl_3$, δ): Biliverdin moiety: 6.88 (1 H, s), 6.65 (1 H, m), 6.53 (1 H, m), 6.17 (1 H, m), 6.09 (1 H, s), 6.04 (1 H, s), 5.67 (2 H, m), 5.48 (1 H, m), 2.96 (4 H, m), 2.60 (2 H, m), 2.45 (2 H, m), 2.20 (3 H, s), 2.13 (3 H, s), 2.09 (3 H, s), 1.91 (3 H, s); acyl substituent: 6.33, 4.48, and 1.32 (5 H, AMX_3, J_{AM} = 7.4 Hz, J_{MX} = 7.2 Hz), 3.67 (3 H, s), 1.46 (9 H, s).

Biliverdin-IXα mono[(S)-aspartic acid dimethyl ester]monomethyl ester: Isomers **18** *and* **19**

From the isomeric mixture of biliverdin-IXα monomethyl esters and (*S*)-aspartic acid dimethyl ester hydrochloride; R_f(**18**) < R_f(**19**).

18: M.p. 134–136 °C, m.s.: 740 ($M+1$), ^{1}H n.m.r. ($CDCl_3$, δ): Biliverdin moiety: 6.82 (1 H, s), 6.63 (1 H, m), 6.53 (1 H, m), 6.16 (1 H, m), 6.08 (1 H, s), 6.03 (1 H, s), 5.68 (2 H, m), 5.47 (1 H, m), 2.96 (4 H, m), 2.63 (2 H, m), 2.48 (2 H, m), 2.20 (3 H, s), 2.12 (3 H, s), 2.09 (3 H, s), 1.90 (3 H, s); acyl substituent: 6.56, 4.81, 2.93, and 2.72 (4 H, AMXY, J_{AM} = 7.5 Hz, J_{MX} = J_{MY} = 4.0 Hz, J_{XY} = 17.4 Hz), 3.70 (3 H, s), 3.66 (3 H, s), 3.63 (3 H, s).

19: M.p. 137–140 °C, m.s.: 740 ($M+1$), ^{1}H n.m.r. ($CDCl_3$, δ): Biliverdin moiety: 6.83 (1 H, s), 6.64 (1 H, m), 6.53 (1 H, m), 6.16 (1 H, m), 6.08 (1 H, s), 6.01 (1 H, s), 5.67 (2 H, m), 5.46 (1 H, m), 2.95 (4 H, m), 2.62 (2 H, m), 2.47 (2 H, m), 2.20 (3 H, s), 2.13 (3 H, s), 2.11 (3 H, s), 1.91 (3 H, s); acyl substituent: 6.58, 4.80, 2.93, and 2.72 (4 H, AMXY, J_{AM} = 8.0 Hz, J_{MX} = J_{MY} = 4.0 Hz, J_{XY} = 17.4 Hz), 3.70 (3 H, s), 3.66 (3 H, s), 3.63 (3 H, s).

Biliverdin-IXα mono[(S)-N(methyl)valine methyl ester]monomethyl ester: Isomers **20** *and* **21**

From the isomeric biliverdin-IXα monomethyl esters and (*S*)-*N*(methyl)valine methyl ester hydrochloride; R_f(**20**) > R_f(**21**).

20: M.p. 81–83 °C, m.s.: 724 ($M+1$), ^{1}H n.m.r. ($CDCl_3$, δ) [15]: Biliverdin moiety: 6.86 (0.75 H, s), 6.85 (0.25 H, s), 6.66 (1 H, m), 6.54 (1 H, m), 6.16 (1 H, m), 6.11 (1 H, s), 6.05 (1 H, s), 5.69 (2 H, m), 5.46 (1 H, m), 3.00 (4 H, m), 2.57 (4 H, m), 2.21 (3 H, s), 2.13 (3 H, s), 2.10 (3 H, s), 1.91 (3 H, s); acyl substituent: 5.00 (0.75 H, d, J = 10.8 Hz), 3.91 (0.25 H, d, J = 10.6 Hz), 3.72 (0.75 H, s), 3.69 (3 H, s), 3.68 (2.25 H, s), 2.93 (2.25 H, s), 2.90 (0.75 H, s), ~2.1 (1 H, m), 1.00 (2.25 H, d, J = 6.5 Hz), 0.94 (0.75 H, d, J = 6.5 Hz), 0.87 (0.75 H, d, J = 6.5 Hz), 0.84 (2.25 H, d, J = 6.5 Hz).

21: M.p. 83–85 °C, m.s.: 724 ($M+1$), ^{1}H n.m.r. ($CDCl_3$, δ) [15]: Biliverdin moiety: 6.85 (0.75 H, s), 6.84 (0.25 H, s), 6.64 (1 H, m), 6.52 (1 H, m), 6.15 (1 H, m), 6.10 (1 H, s), 6.04 (1 H, s), 5.68 (2 H, m), 5.46 (1 H, m), 3.00 (4 H, m), 2.58 (4 H, m), 2.21 (3 H, s), 2.13 (3 H, s), 2.10 (3 H, s), 1.91 (3 H, s); acyl substituent: 4.99 (0.75 H, d, J = 10.7 Hz), 3.90 (0.25 H, d, J = 10.7 Hz), 3.70 (0.75 H, s), 3.68 (3 H, s), 3.66 (2.25 H, s), 2.94 (2.25 H, s), 2.90 (0.75 H, s), ~2.1 (1 H, m), 0.99 (2.25 H, d, J = 6.5 Hz), 0.94 (0.75 H, d, J = 6.5 Hz), 0.89 (0.75 H, d, J = 6.5 Hz), 0.84 (2.25 H, d, J = 6.5 Hz).

References and Notes

[1] Part 1: *Haidl H, Krois D, Lehner H* (1985) J Chem Soc Perkin 2: 421

[2] For reviews see: *Scheer H* (1981) Angew Chem 93: 230; Angew Chem Int Ed Engl 20: 241 (1981); *Goodwin WT* (1976) In: Chemistry and biochemistry of plant pigments, 2nd edn. vol 1. Academic Press, New York, p 328

[3] *Haidl H, Krois D, Lehner H* (1985) Monatsh Chem 116: 119

[4a] *Falk H, Kapl G, Medinger W* (1985) Monatsh Chem 116: 1065

[4b] *Falk H, Thirring K* (1981), Tetrahedron 37: 761

[5] Thus, the $\Delta\varepsilon$-values of the (*R*)-phenethylamin derivative in Ref. [4a] lacking the second coordination site are low. On the other hand chiral induction in the (*S*)-alanine and (*S*)-phenylalanine compounds are large; their $\Delta\varepsilon$-values closely resemble those obtained for a monoalanine derivative of biliverdin-XIIIα [1]

[6] Biliverdin (*S*)-amino acid amides will conveniently be considered in connection with bilipeptides in a forthcoming paper [11]

[7] Diastereoisomeric amino acid derivatives of 2,3-dihydrobilatriene-abc-3-acetic acid are less suited to study the influence of structural variations. This is due to the presence of an additional chirality centre within ring A. Although not claimed explicitly in Ref. [4a] the influence of this chirality centre on chiral discrimination of the bilatriene helices might exceed that of the amino acid residue in the side chain. Moreover, this influence should strongly depend on the nature of the ligands

[8] *Wagnière G, Blauer G* (1976) J Amer Chem Soc 98: 7806; *Falk H, Höllbacher G* (1978) Monatsh Chem 109: 1429

[9] We restrict our discussion to the long wave length c. d.-band at λ ca. 660 nm. The same conclusions are arrived at if the blue band at λ ca. 380 nm is considered

[10] The relationship between the discriminating free enthalpy ΔG and the enantiomeric excess (ee) is almost linear if ee does not exceed 50%. Since for the enantiomeric homogeneous bilatriene helix a $\Delta\varepsilon$-value of ca. $\pm$ 100 (long wave length band) seems plausible [4b, 11] the demand for the additivity of $\Delta\varepsilon$-values of the individual side chains in biliverdin bis(amino acids) is justified. In this context the term *enantiomeric excess* is used with reference to the enantiomeric bilatriene helices present in *two diastereoisomers*

[11] *Krois D, Lehner H*, J Chem Soc Perkin 2, in press

[12] *Lehner H, Braslavsky S, Schaffner K* (1978) Ann Chem 1978: 1990

[13] *Deimer K-H* (1974) In: *Wünsch E* (ed) Houben Weyl, vol 15/1, 4th edn. Thieme, Stuttgart

[14] *Shemyakin MM, Ovchinnikov YuA, Kiryushkin AA, Ivanov VT* (1962) Izv Akad Nauk SSSR Otd Khim Nauk 2154; CA. 58: 14087

[15] Mixture of rotamers

[16] *Poroshin KT, Shibnev VA, Kozarenko TD* (1959) Izv Akad Nauk SSSR Otd Khim Nauk 736; CA. 53: 21693

[17] *Grassmann W, Wünsch E* (1958) Chem Ber 91: 449