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Reactivity of Pyrrole Pigments: Part 16.[1] Mesobiliverdin IXα and Mesobilirubin IXα Bridged between the Propionic Acid Substituents

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Abstract.: Mesobiliverdin IXα diesters of α,ω-dialkanols were obtained by reaction of mesobiliverdin IXα dicesium salt with diiodomethane, 1,3-dibromopropane and 1,4-dibromobutane. Mesobiliverdin IXα methylene diester was tested and compared to mesobiliverdin IXα dimethyl ester, for their photoisomerization behaviour, addition of nucleophiles at C10, protonation, and deprotonation. The constriction imposed by the internal cycle to the flexibility around the two central rings has no appreciable effect on these reactions. In fact, the small pK_a differences in the protonation indicate that previous results, on the protonation of biliverdins bridged between the end rings, can be explained through a stretched structure of the protonated bile pigment implying a change in the configuration or conformation of one end ring.

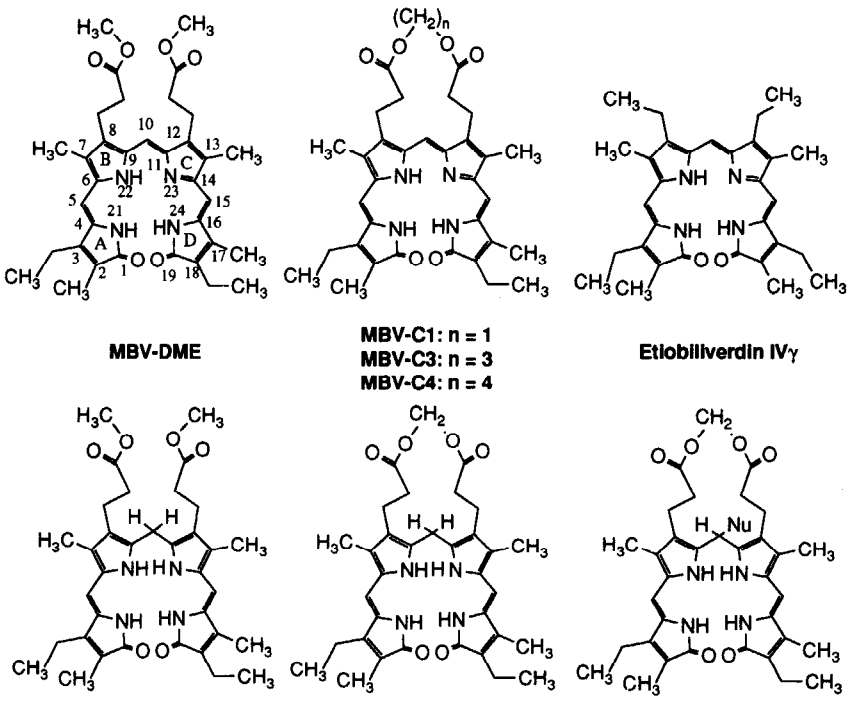
Introduction

The linear pyrrole pigment in biliproteins can adopt conformations and configurations that are different from the more stable ones found in solution.[2] Studies of solutions of bile pigments with fixed geometry can be used to infer the role of bile pigment structure in the photophysical and photochemical responses of biliproteins.[2] The flexibility constriction imposed by bridging end rings A and D has been performed (N21-N24 methylene bridged) to study the structure of biliverdins (bilatrienes-*abc*).[3] A stilbene bridge has been used to study photoisomerization processes in biliverdins;[4] 2,18-bridged biliverdins have been synthesised.[5] Marked differences with open biliverdins have been reported in the protonation of these bridged derivatives.[6]

One of the flexibility constrictions expected on linear tetrapyrroles in biliproteins is that caused by the interaction of the propionic substituents of the bile pigment with the basic aminoacids of the protein.[7] Biliverdins bridged *via* the propionic acid substituents may mimic this flexibility constriction in biliproteins. However, the synthesis of suitable models for the flexibility constriction at C10 has not been reported, although failed attempts have been described,[8] based on the synthesis of internal cycled biliverdins using the propionic acid substituents at C8 and C12.

Internally cyclised bilatrienes-*abc* such as **MBV-C1** of Formula Scheme would show structures with *Z-syn* configuration-conformation at C10 as the most stable. The *E* configuration at C10 would be less accessible than that corresponding to **MBV-DME**. Furthermore, the *anti* conformations at C10 would be unattainable.[9] Internal cycled bilirubins (biladienes-*ac*) such as **MBR-C1** would show ridge-tile or, near to orthogonality, dipyrrinone halves with *syn* configuration around C10 (*anti* for the non cyclised **MBR-DME**).[2a,e]

We now present the synthesis of biliverdins cyclised through an internal diester group between the propionic substituents at C8 and C12, together with an evaluation of the differences in chemical behaviour



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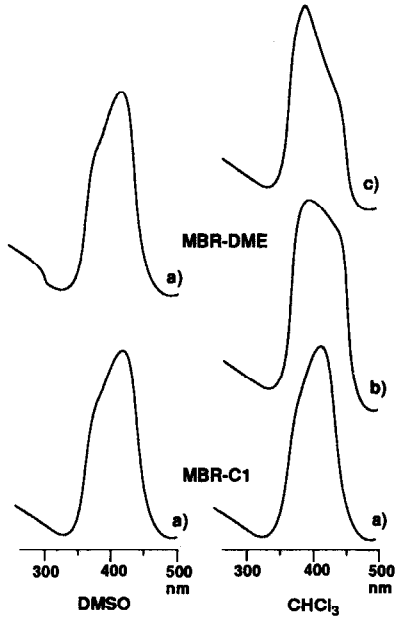

Figure 2. UV/Vis absorption spectra in DMSO and $CHCl_3$ of MBR-C1 compared to MBR-DME. ***a)*** *spectra do not change with concentration (range between 2 10^{-6} and 5 10^{-5} mol l^{-1}).* ***b)*** *2 10^{-6}mol l^{-1}.* ***c)*** *5 10^{-5} mol l^{-1}.*

between the bridged **MBV-C1** and the non-bridged **MBV-DME** (mesobiliverdin IXα dimethyl ester) systems [addition of nucleophiles to C10 (**MBRub-C1**): photoisomerization (Z,Z,Z == E,Z,Z), protonation and deprotonation]. The first results on the cyclised mesobilirubin **MBR-C1** obtained by reduction from **MBV-C1** are also presented.

Results and Discussion

Synthesis of mesobiliverdin IXα alkandiyl esters

The possibility of synthesising the internal diesters of C8 and C12 propionic acid substituted biliverdins was suggested by the ease with which bile pigments could be anchored in functionalized, non-crosslinked polystyrenes through the reaction of the bile pigment dicesium salts upon electrophilic centres in the functionalized polymer.[10]

The title internal esters were obtained by reaction, in an aprotic solvent at high dilution, of the dicesium salt of mesobiliverdin IXα with an α,ω-dihalogenated n-alkane. DMSO was a better solvent than DMF, mainly because of the presence of small amounts of dimethylamine in the latter, which reacted with the final esters, owing to the high dilution conditions and the long reaction times. The experimental procedure involves the addition of the dihalogenated derivative (≈ 0.01 mol l^{-1}) to a solution of the dicesium salt of mesobiliverdin IXα (≈ 1 10^{-4} mol l^{-1}). This addition should be slow, and the yields increase significantly if it is performed in fractions separated by sufficient intervals to allow the reaction to take place. In these conditions (see Experimental) a 46 % yield was obtained for the acylal **MBV-C1** by four slow additions separated by 24 hours. The limiting factor for the yield is the difficulty in maintaining anhydrous conditions for so long. The work-up procedure should be performed in such a way as to prevent the formation of elemental halogen from the dihalogenated alkane: bile pigments are oxidized by halogens.[1] Thus **MBV-C2** could not be obtained because the easy formation of elemental halogen from the initial 1,2-diiodoethane. However, **MBV-C3** and **MBV-C4** were obtained in ≈20% yield from the corresponding α,ω-dibromoalkane.

^{1}H-NMR spectra of the cyclic esters are very similar to that of **MBV-DME**. As expected, NOE and COSY relationships are similar to that of the open structure, which have the helical *Z,Z,Z-syn,syn,syn* structure.[11] The UV/Vis spectra of **MBV-DME** and those of the internal esters are also similar (see Fig. 1).

The chemical properties of the internal esters were compared with those of the non-constricted **MBV-DME**. There is a difference only in the case of **MBV-C1**, which was expected to exert a higher effect on the mobility around C10, since its cycle is smaller than that of **MBV-C3** or **MBV-C4**.

Photoisomerization of the exocyclic double bonds at C4 or C16.

In parallel experiments the photoisomerization of **MBV-DME** and **MBV-C1** were compared. The simple procedure for photoisomerization of bilatrienes-*abc* was followed.[12] The initial products show the *Z,Z,Z* configuration exclusively, but after photoisomerization procedure, a mixture of the initial configuration (≈ 70%) and the *E,Z,Z* and *Z,Z,E* isomers (1:1) was obtained.[12] By TLC, no significant differences were observed between the experiments with the open diester and those with the cyclic diester.

Photoisomerization of bilatrienes-*abc* involves the reversible addition of a nucleophile at C10 to give a bilirubinoid (biladiene-*ac*), which is in fact the substrate for the photoisomerization.[12] In this respect,

differences in chemical behaviour between **MBV-DME** and **MBV-C1** should be detected more clearly in the experiments in which nucleophiles are added at C10 (see below).

Protonation

Dilute solutions of **MBV-DME** or **MBV-C1** in $CHCl_3$ show, by addition of *p*-toluensulfonic acid, the same behaviour. Addition of acid gives UV/Vis spectra with the same shape and pattern for both bilatrienes (Fig. 1). The shoulder above 720 nm can be attributed to aggregated species, because it becomes more marked at higher concentrations of bilatriene. Nevertheless, the spectrometric titration of a $CHCl_3$ solution ($3\ 10^{-6}$ mol l^{-1}) with a saturated $CHCl_3$ solution of *p*-toluensulfonic acid gives a set of spectra which in spite do not show "pure" isosbestic points, allow estimation of the differences in basicity between the bilatrienes. The protonation of **MBV-DME** is easier than that of **MBV-C1,** but the ΔpK_a is only ≈ 0.2, *i. e.* there is only a very small effect of the constriction at C10.

This result is in contrast with the high effect on the pK_a performed by the bridging between the rings A and D.[6] Such bridging results in a decrease of the basicity of several orders of magnitude ($\Delta pKa \approx 3.4$), and the protonated form shows a spectrum with different shape and pattern than the spectrum of the open bilatriene.[6]

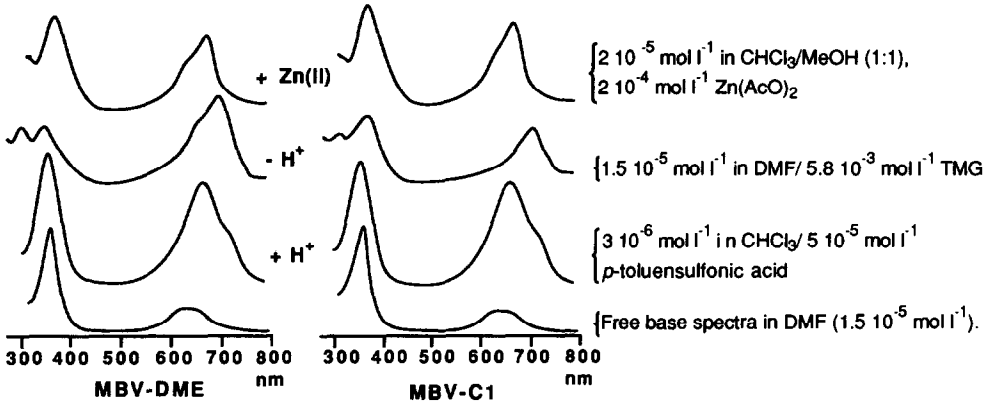

Figure 1. UV/Vis absorption spectra of ***MBV-C1*** *compared to* ***MBV-DME****.*

The bilatriene-*abc* protonation side is the nitrogen of the pyrrolenine fragment [2a,e]. The structure of the protonated bilatriene-*abc* must be stretched.[6] Further our results rule out the possibility that the protonation performs a strong structural change around C10, and point to a change around C5 or C15. In fact, protonated dipyrrins (the partial model of the two central rings of biliverdins) retain de *Z-syn* structure of the free-base dipyrrin: only an increase in the dihedral angle between rings can be expected.[13] Therefore, in this sense, our results agree with this model: the constriction imposed in **MBV-C1** to C10 does not have much influence on the pK_a of the protonation process on the central dipyrrin unit (rings B and C). A structural change on C5 or

C15 due to the protonation at N23 could be explained through the suppression of the internal hydrogen bonds between the NH groups and the pyrrolenine N [2e] and the change from partial dipole interactions between rings[14] to another type of electrostatic interaction.

Deprotonation

The spectrophotometric titration, with tetramethylguanidine (TMG) as a base, of **MBV-DME** and **MBV-C1** in DMSO or DMF give isobestic points in all cases. The spectrophotometric titration of **MBV-DME** in these solvents and with TMG has been described[15] but the absolute pK_a values could not be determined because of the lack of the activity coefficient data for these solvent systems. However, the difference in the log[TMG], at $log[AH]/[A^-] = 1$, shows a value of 0.54±0.03 in DMSO and in DMF: (**MBV-DME** is easier to deprotonate than **MBV-C1**), *i. e.* ΔpK_a of this order. The UV/Vis spectra of the conjugated base show similar shape and pattern (see Fig. 1) but lower absorption and higher red-shift of the low energy band in the case of **MBV-C1** (26 nm in DMF and 34 nm in DMSO). This small $\Delta\Delta pK_a$ value[16] indicates that the constriction of C10 does not affect the NH deprotonation.[17] The small differences between the spectra of the two conjugated bases suggest that their structures are slightly different.

The complexes of **MBV-DME** and **MBV-C1** with Zn(II), whose formation involves the deprotonation of the bilatriene-*abc* and the formation of helical complexes around the metal ion,[18] show very similar UV/Vis spectra (see Fig. 1), *i. e.* similar structure in the complex of both biliverdins and no significant effect of the C10 constriction. In fact, the Zn(II) complex spectra are similar to those of the conjugated bases in DMF or DMSO,[19] which points to a similar structure of the deprotonated ligand in both cases. Probably the template effect in the complex of the metal cation results in a more similar structure for both ligands than in the free conjugated base form. The quantum yield for the fluorescence spectra of **MBV-C1-Zn(II)** is about 5 times higher than for **MBV-DME-Zn(II)**, which agrees with a more rigid structure for the former.

*Internally cyclised biladienes-ac (**MBR-C1** and adducts **MBRub-C1**) obtained by the addition of nucleophiles at the C10 position of **MBV-C1**.*

In $CHCl_3$, *tert*-butyl amine or piperidine, at an excess to bile pigment of 10000:1, did not add either to **MBV-DME** or to **MBV-C1** (UV/Vis). Nevertheless, in the same conditions etiobiliverdin IVγ adds piperidine but not *t*-butylamine, in agreement with already reported results.[20] This different behaviour of **MBV-DME** and **MBV-C1** compared to etiobiliverdin IVγ must be a consequence of a higher steric hindrance effect of the propionic substituent compared to the ethyl. In the case of a small nucleophile, such as mercaptoethanol, the adduct is formed in both cases but differences are observed which show a slightly higher steric hindrance effect for the internal ester **MBV-C1** than for **MBV-DME**.

Table 1 shows the differences on the mercaptoethanol addition to **MBV-DME** and **MBV-C1**. The equilibrium constants for **MBV-C1** and **MBV-DME** have values of one at approximately 325 K and 350 K in $CHCl_3$ and at 365 K and 400 K in DMSO. Although, due to the errors in the measurement of the mercaptoethanol concentration (variations in the disulphur content), absolute equilibrium constant, and consequently $\Delta G°$ values, are difficult to determine. However, the $\Delta\Delta G°$, $\Delta\Delta H°$ and $\Delta\Delta S°$ values show, as expected, an enthalpy difference in favour of the addition of the less hindered **MBV-DME** in $CHCl_3$ as well in DMSO, but a $\Delta\Delta S°$ due to a less negative entropy for the addition to the cyclic **MBV-C1**.

*Table 1. Gibbs Energy, Enthalpy and Entropy Differences (**MBV-DME - MBV-C1**) for the Equilibrium Reaction with Mercaptoethanol in $CHCl_3$ ($1.5\ 10^{-5}$ mol l^{-1} substrate and 0.15 mol l^{-1} mercaptoethanol) and DMSO ($1.1\ 10^{-2}$ mol l^{-1} substrate and 0.15 mol l^{-1} mercaptoethanol). $CHCl_3$ Experiments Were Followed by UV/Vis Spectrometry and DMSO Experiments by ^{1}H-NMR.*

$HCCl_3$ [a]				DMSO [b]			
T (K)	ΔΔG° (kJ mol^{-1})	ΔΔH° (kJ mol^{-1})	ΔΔS° (J mol^{-1} l^{-1})	T (K)	ΔΔG° (kJ mol^{-1})	ΔΔH° (kJ mol l^{-1})	ΔΔS° (J mol^{-1} l^{-1})
298	-5.0±0.1			340	-12.1±0.1		
		-21±1	-51±3			-28±1	-48±3
328	-4.6±0.1			400	-9.3±0.1		

a) determined in the range between 288K and 328K

b) determined in the range between 298K and 400K

Rotational, vibrational or conformational entropy contributions would not explain this entropy difference because they must be more positive for the adduct **MBRub-DME**. The results described below for **MBR-C1** suggest that this ΔΔS° could be attributed to the absence of aggregation (see below).

Reduction of **MBV-C1** with $NaBH_4$ in methanol at room temperature gives **MBR-C1**. The UV/Vis spectrum of **MBR-C1** does not change with the concentration either in DMSO or in $CHCl_3$ (see Fig. 2). Furthermore, the same shape is observed for the spectra in DMSO or $CHCl_3$. This shape could be due to an exciton splitting with a more intense red shifted band. These results are in contrast with the behaviour of **MBR-DME**. **MBR-DME** is dimeric in $CHCl_3$ in non-diluted solutions, and it is monomeric in very diluted (<1 10^{-6} mol l^{-1}) $CHCl_3$ solutions: further, the monomeric form in DMSO shows a different spectrum (red shifted), *i. e.* a spectrum very similar to **MBR-C1** in the same solvent. The UV/Vis spectrum of bilirubins has been studied extensively.[2a,e] For its interpretation the exciton coupling model of the dipyrrinone halves is frequently used.[21] However, in addition to the stereo chemical structure of the biladiene-*ac*, the presence or not of intermolecular aggregation and the structure of these aggregates must be determined. Aggregation of bilirubins, in non-protic solvents, is originated by the formation of intermolecular hydrogen bonds between the dipyrrinone halves of the biladiene-*ac*, which also results in exciton splitting.[22] The spectra of **MBR-C1** would agree with a non-aggregated *syn* form (torsion angles around C10 of ≈ +60° and -60°), which results in an exciton coupling with a more intense red-shifted band.[23] In this respect, work is in progress.

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Experimental

The preparation of mesobiliverdin IXα dimethyl ester (**MBV-DME**) from bilirubin IXα (Janssen Chimica) is described in the literature.[24]

Thin layer chromatography (TLC) analysis was performed on silica gel with chromatofoils (DC-Alufolien F_{254}, Merck). Preparative thin layer chromatography (PTLC) was performed on 1mm thick silica gel (Merck) plates without fluorescence indicator.

Melting points were determined on a Kofler-Reichert microhot stage apparatus. UV/Vis spectra were recorded on a Perkin-Elmer Lambda 5 instrument. MS on a Hewlett-Packard 5988A instrument equipped for FAB analysis with a Capillaritron Frasor (Xe). ^{1}H-NMR spectra were determined on a Geminis 200 Varian (200 MHz) instrument: chemical shifts are referred to TMS as internal reference.

Photoisomerization tests were performed in parallel experiments with ≈ 1 mg of substrate (**MBV-DME** and **MBV-C1**) in 0.3 g neutral Al_2O_3 according the experimental procedure described in [11a]: photoisomerization products were detected by TLC: on Al_2O_3 chromatofoils, $CHCl_3$/MeOH (90:1), R_f: for **MBV-DME**, *ZZZ* 0.80, *EZZ* and *ZZE* 0.24 and 0.20; for **MBV-C1**, *ZZZ* 0.75, *EZZ* and *ZZE* 0.27 and 0.21.

For the nucleophile addition at C10 experiments, 1.5 10^{-5} mol l^{-1} solutions of the substrate in $CHCl_3$, with the nucleophile, were observed for the UV/Vis spectra changes with the temperature (15°-55°C) by UV/Vis. In the case of the ^{1}H-NMR experiments, DMSO-d_6 solutions of the substrate (1.2 10^{-2} mol l^{-1}) and mercaptoethanol (1 mol l^{-1}) were observed for the changes on the range of temperatures between 25°-135°C. All experiments were reversible to the temperature changes.

Deprotonation experiments were performed in 1.5 10^{-5} mol l^{-1} solutions in DMSO or DMF as solvents using 1,1,3,3-tetramethylguanidine (TMG). The ratio between bilatriene-*abc* and deprotonated bilatriene-*abc* was followed by UV/Vis. For more experimental details and on the calculation of species ratio see ref. [15].

Mesobiliverdin IXα methylene ester (MBV-C1)

The dicesium salt of mesobiliverdin IXα was obtained from the addition of an equimolecular amount of cesium carbonate solution in water (0.06 mol l^{-1}) to a 3 mmol l^{-1} mesobiliverdin IXα solution in DMSO: the solution was evaporated and dried under vacuum. In anhydrous conditions, with magnetic stirring and under Argon atmosphere, 2.3 ml of a solution 7.7 10^{-2} mol l^{-1} CH_2I_2 in DMSO it were added to 0.1 mmol of the dicesium salt of mesobiliverdin IXα dissolved in 70 ml DMSO. The addition was performed in four fractions, drop-by-drop (12 h) and the mixture was left to react for 36 h (total reaction time 8 days). The reaction mixture was added to 200 ml $CHCl_3$, washed several times with water, dried over anhydrous Na_2SO_4 and vacuum evaporated. The crude product was purified by PTLC using $CHCl_3$/CH_3OH (9:1). The most mobile band (R_f = 0.7) was isolated and purified by column chromatography on a small silica column: 28 mg (46 %). M.p. >300°C.

MS (FAB, Xe, *m/z*): 599 (M+1).

UV/Vis [λ_{max} *nm* (ε)]: $CHCl_3$: 628 (12000), 367 (44000). DMF; 631 (13700), 368 (48400). DMSO; 630 (12500), 369 (46200).

^{1}H-NMR ($CDCl_3$, δ, *ppm*): 8.25 (broad s, NH), 6.69 (s, C10, =CH), 5.90 and 5.89 (2 s, C5 and C15, =CH), 5.79 (s, O-CH_2-O), 2.93 (t, -$\underline{CH_2}$-CH_2-COO-), 2.64 (t, -CH_2-$\underline{CH_2}$-COO-), 2.52 (q, C3, -$\underline{CH_2}$-CH_3), 2.28 (q, C18, -$\underline{CH_2}$-CH_3), 2.10 (s, 6H, C7 and C13, -CH_3), 2.08 (s, C17, -CH_3), 1.83 (s, C2, -CH_3), 1.21 (t, C3, -CH_2-$\underline{CH_3}$), 1.08 (t, C18, -CH_2-$\underline{CH_3}$).

Conjugated base:

UV/Vis [λ_{max} *nm* (ε)]: DMF/TMG; 320 (27000), 374 (34000), 670 sh (16000), 722 (21500). DMSO/TMG; 320 (25000), 381 (31000), 680 sh. (15000), 730 (23000).

Zn(II) complex:

2.5 mol l^{-1} solutions in $CHCl_3$/CH_3O (1:1) 2 10^{-4} mol l^{-1} Zn diacetate.UV/Vis [λ_{max} *nm* (ε)]: 682 (33700), 630 sh., 368 (40400); λ_{em} = 706 nm.

Conjugated acid:

UV/Vis [λ_{max} *nm* (ε)]: 3 10^{-6} mol l^{-1}, $CHCl_3$/ 45 10^{-6} *p*-toluensulfonic acid; 720 sh, 650 (39000), 364 (49000).

Spectral data for ***MBV-DME***

In the same experimental conditions as for **MBV-C1**:

UV/Vis [λ_{max} *nm* (ε)]: $CHCl_3$; 632 (14800), 367 (54700). DMF; 634 (16200), 367 (51600). DMSO; 631(15400), 368 (49600).

Conjugated base:

UV/Vis [λ_{max} *nm* (ε)]: DMF/TMG; 324 (28200), 370 (27500), 644 sh (26800), 702 (47100). DMSO/TMG; 325 (26700), 376 (26100), 646 sh. (21200), 705 (41500).

Zn(II) complex: λ_{max} *nm* (intensity ratio): 685 (28000), 632 sh., 370 (39500); λ_{em} = 711 *nm*.

Conjugated acid:

UV/Vis [λ_{max} *nm* (ε)]: 3 10^{-6} mol l^{-1}, $CHCl_3$/ 45 10^{-6} *p*-toluensulfonic acid; 720 sh, 654 (35500), 364 (47400).

Mesobiliverdin IXα propan-1,3-diyl ester (MBV-C3).

In anhydrous conditions, with magnetic stirring and under Argon atmosphere, 0.5 ml of a solution 3.9 10^{-2} mol l^{-1} of 1,3-dibromopropane in DMSO was added to 10 mg (0.016 mmol) of the dicesium salt of mesobiliverdin IXα dissolved in 25 ml DMSO. The addition was performed drop-by-drop in fractions of 0.1 ml each 12 h and the mixture was left to react between additions (total reaction time 7 days). The reaction mixture was added to 150 ml $CHCl_3$, washed several times in water, dried over anhydrous Na_2SO_4 and vacuum evaporated. The crude product was purified by PTLC using $CHCl_3$/CH_3OH (9:1). The most mobile band (R_f = 0.7) was isolated and purified by column chromatography on a small silica column: 3 mg (19 %). M.p. >300°C.

MS (FAB, Xe, *m/z*): 627 (M+1).

^{1}H-NMR ($CDCl_3$, d, *ppm*): 6.88 (s, C10, =CH), 5.89 and 5.88 (2 s, C5 and C15, =CH), 4.27 (t, 4H, O-$\underline{CH_2}$-CH_2-$\underline{CH_2}$-O), 2.93 (t, 4H, -$\underline{CH_2}$-CH_2-COO-), 2.64 (t, 4H, -CH_2-$\underline{CH_2}$-COO-), 2.52 (q, C3, 2H, -$\underline{CH_2}$-CH_3), 2.28 (q, C18, 2H, -$\underline{CH_2}$-CH_3), 2.10 (s, 6H, C7 and C13, -CH_3), 2.08 (s, C17, -CH_3), 1.83 (s, C2, -CH_3), 1.25 (q, 2H, O-CH_2-$\underline{CH_2}$-CH_2-O), 1.21 (t, C3, -CH_2-$\underline{CH_3}$), 1.09 (t, C18, -CH_2-$\underline{CH_3}$).

Mesobiliverdin IXα butan-1,4-diyl ester (MBV-C4).

In anhydrous conditions, with magnetic stirring and under Argon atmosphere, 0.65 ml of a solution 3.9 10^{-2} mol l^{-1} of 1,4-dibromobutane in DMSO was added to 10 mg (0.016 mmol) of the dicesium salt of mesobiliverdin IXα dissolved in 25 ml DMSO. The addition was performed drop-to-drop in 0.13 ml fractions each 12 h and the mixture was left to react between additions (total reaction time 7 days). The reaction mixture was added to 150 ml $CHCl_3$, washed several times with water, dried over anhydrous Na_2SO_4 and vacuum evaporated. The crude product was purified by PTLC using $CHCl_3/CH_3OH$ (9:1). The most mobile band (R_f = 0.7) was isolated and purified by column chromatography on a small silica column: 3 mg (20 %). M.p. >300°C.

MS (FAB, Xe, *m/z*): 641 (M+1).

^{1}H-NMR ($CDCl_3$, δ, *ppm*): 6.88 (s, C10, =CH), 5.89 and 5.88 (2 s, C5 and C15, =CH), 4.16 (t, 4H, O-$\underline{CH_2}$-CH_2-CH_2-$\underline{CH_2}$-O), 2.93 (t, 4H, -$\underline{CH_2}$-CH_2-COO-), 2.64 (t, 4H, -CH_2-$\underline{CH_2}$-COO-), 2.52 (q, C3, 2H, -$\underline{CH_2}$-CH_3), 2.28 (q, C18, 2H, -$\underline{CH_2}$-CH_3), 2.10 (s, 6H, C7 and C13, -CH_3), 2.08 (s, C17, -CH_3), 1.83 (s, C2, -CH_3), 1.25 (q, 2H, O-CH_2-$\underline{CH_2}$-$\underline{CH_2}$-CH_2-O), 1.21 (t, C3, -CH_2-$\underline{CH_3}$), 1.08 (t, C18, -CH_2-$\underline{CH_3}$).

Mesobilirubin IXα methylene ester (MBRC1)

To a solution of 10 mg (0.017 mmol) of **MBV-C1** in 20 ml methanol it was added, just to the conversion of the biliverdin in bilirubin colour, drop-by-drop at room temperature a 1.7 10^{-2} mol l^{-1} solution of $NaBH_4$ in methanol . Addition of 200 ml $CHCl_3$ and washing with water affords, after drying with Na_2SO_4 and evaporation of the organic phase, the crude **MBR-C1**, which was purified by TLC (3.8 mg, 36 %).

MS (FAB, Xe, *m/z*): 601 (M+1).

UV/Vis [λ_{max} *nm* (ε)]: $CHCl_3$: 416, 395 sh.. DMSO; 426, 394 sh..

^{1}H-NMR ($CDCl_3$, δ, *ppm*): 10.64 (broad s, NH), 9.25 (broad s, NH), 6.07 (broad s, centre AB system O-CH_2-O), 6.00 and 5.96 (two s, C5 and C15 =CH), 2.82 (center broad m, 8H, -$\underline{CH_2}$-$\underline{CH_2}$-COO-), 2.49 (q, C3, -$\underline{CH_2}$-CH_3), 2.32 (q, C18, -$\underline{CH_2}$-CH_3), 2.13 and 2.07 (two s, 6H, 3H, C7, C13, and C17 -CH_3), 1.86 (s, C2, -CH_3), 1.13 (t, C3, -CH_2-$\underline{CH_3}$), 1.06 (t, C18, -CH_2-$\underline{CH_3}$).

References

1. Part XV: Acero, C.; Ribó, J. M.; Solé, R.; Trull, F. R. *Monatsh. Chem.* **1993**, *124*: 401-417.
2. a) McDonagh A. F.: Bilatrienes and 5,15-Biladienes. In *The Porphyrins* (Dolphin D. Ed.), Vol. 6, part A, Academic Press, New York, 1979; pp. 293-491. b) Rüdiger W. *Structure and Bonding* (Berlin) **1980**, *40*, 101. c) Scheer H. *Angew. Chem. Int. Ed.* **1981**, *20*, 241-261. d) Braslavsky S. E. (1984) *Pure and Appl. Chem.* **1984**, *56*: 1153-1165. e) Falk H. *The Chemistry of Linear Oligopyrroles and Bile Pigments* Springer, Wien-New York, 1989.
3. Falk, H.; Thirring, K. *Tetrahedron* **1981**, *37*, 761-766.
4. Nesbadva, P.; Gossauer, A. *J. Amer. Chem. Soc.* **1987**, *109*, 6545-6546.
5. a) Krois, D.; Lehner, H. *J. Chem. Soc. Perkin Trans. I*, **1989**, 2179-2185. b) Krois, D.; Lehner, H. *J. Chem. Soc. Perkin Trans. 2*, **1993**, 1351-1360. c) Sturrock, E. D.; Bull, J. R.; Kirsch, R. E.; Pandey, R. K.; Senge, M. S.; Smith, K. M. *J. C. S. Chem. Comm.* **1993**, 872-874.
6. Krois, D *Monatsh. Chem.* **1991**, *122*, 495-506
7. Falk, H.; Marko, H.; Müller, N.; Schmitzberger, W.; Stumpe, H. *Monatsh. Chem.* **1990**, *121*, 893-901.
8. Schneider, D. *Modelsubstanzen für den Phytochrom-Chromophor*, Max-Plank-Institut für Strahlen Chemie, Ruhr-Universität Bochum, 1988.
9. Shown using molecular models and computer based molecular modelling systems.
10. Castán, P.; Giralt, E.; Perez, J. C.; Ribó, J. M.; Siscart, N.; Trull, F. R. *Tetrahedron* **1987**, *43*, 2593-2608.
11. Falk, H.; Grubmayr, K.; Magauer, K.; Müller, N.; Zrunek, U. *Israel J. Chem.* **1983**, *23*, 187-194.
12. a) Falk, H.; Grubmayr, K. *Angew. Chem.* **1977**, *89*, 487. b) Falk, H.; Grubmayr, K.; Haslinger, E.; Schlederer, T.; Thirring, K. *Monatsh. Chem.* **1978**, *109*, 1451-1473.
13. Falk, H.; Hofer, O. *Monatsh. Chem.* **1975**, *106*, 115-120.
14. Falk, H.; Ribó, J. M. *Monatsh. Chem.* **1976**, *107*, 307-315.
15. Anglada, C.; Claret, J.; Crusats, J.; Farrera, J.-A.; Ribó, J. M.; Trull, F. R. *Monatsh. Chem.*. **1990**, *121*, 653-663.
16. *e. g.* an ΔpK_a of 0.7 has been reported between 3,18 ethyl or vinyl substitution [ref. 14].
17. the chemical constitution of these conjugated bases has already been demostrated [ref. 2a]
18. Falk, H.; Schlederer, T. *Liebigs Ann. Chem.* **1979**, 1560-1570.
19. similar shape and intensity ratio between bands but smaller red-shifts of the low energy band.
20. Falk, H.; Müller, N.; Schlederer, T. *Monatsh. Chem.* **1980**, *111*, 159
21. Lightner, D. A.; Gawronski, J. K.; Wijekoon, W. M. D. *J. Am. Chem. Soc.* **1987**, *109*, 6354-6362.
22. Falk, H.; Grubmayr, K.; Höllbacher, G.; Hofer, O.; Leodolter, A.; Neufingerl, F.; Ribó, J. M. *Monatsh. Chem.* **1977**, *108*, 1113-1130.
23. The α state would be red shifted, because angles between transition moments and distance vector are around 80° and 100° and between transition moments 30° ($\cos\theta - 3\cos\phi_1\cos\phi_2 > 0$)[see ref. 21].
24. McDonagh, A. F.; Palma, L. A. *Biochem J.* **1980**, *189*, 193-208.

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