Monatshefte für Chemie 136, 755–761 (2005)
DOI 10.1007/s00706-004-0269-4

Monatshefte für Chemie
Chemical Monthly
Printed in Austria

Inducing Geometrical Changes of Biliverdin Chromophores by 23*N*-Methylation

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Received August 29, 2004; accepted October 1, 2004
Published online February 11, 2005

Summary. Transfer of sterical hindrance from the periphery to the center of biliverdins by placing a methyl group at N23 and hydrogens at the β-carbons in position 12 and 13 changes the conformation of the chromophore from (10*syn*,14*syn*) to (10*anti*,14*anti*). Additional reduction of sterical hindrance by placing a further hydrogen at the β-carbon in position 8 induces a change in configuration from (9*Z*) to (9*E*).

Keywords. Bilin-1,19-dione; Chromophores; Configuration; Conformation; NMR spectroscopy.

Introduction

In the preceding paper we have reported on a modular concept to change the conformation of biliverdin chromophores by modifying the size of sterical hindrance and varying the number of intramolecular NH···N hydrogen bonds [1]. Accordingly, single *syn*/*anti* transitions within one of two pyrromethenone moieties or within the dipyrrin substructure could be induced by the replacement of a definite β-substituent with hydrogen in combination with the use of a polar aprotic solvent. Based on this experience biliverdin chromophores *N*-methylated at the dipyrrin subunit were chosen next to increase the number of *anti*-conformers.

With reference to the *anti*-conformers of the *N*-methyldipyrrinone **3** [2] and the *N*-methyltripyrrinone **6** [3] a double-*anti*-conformation of the biliverdin **1** should result from a simple superposition of their pyrrolic subunits in common compared to the *syn*-conformations of their non-*N*-methylated analogues **B** [4, 5], **C** [6], and **A** [7–9] (Scheme 1). Thus our idea to modify the conformation of biliverdin chromophores predominantly rests on the interchange of small hydrogens and bulkier methyl groups. Methyl groups should be found at the periphery of the chromophore

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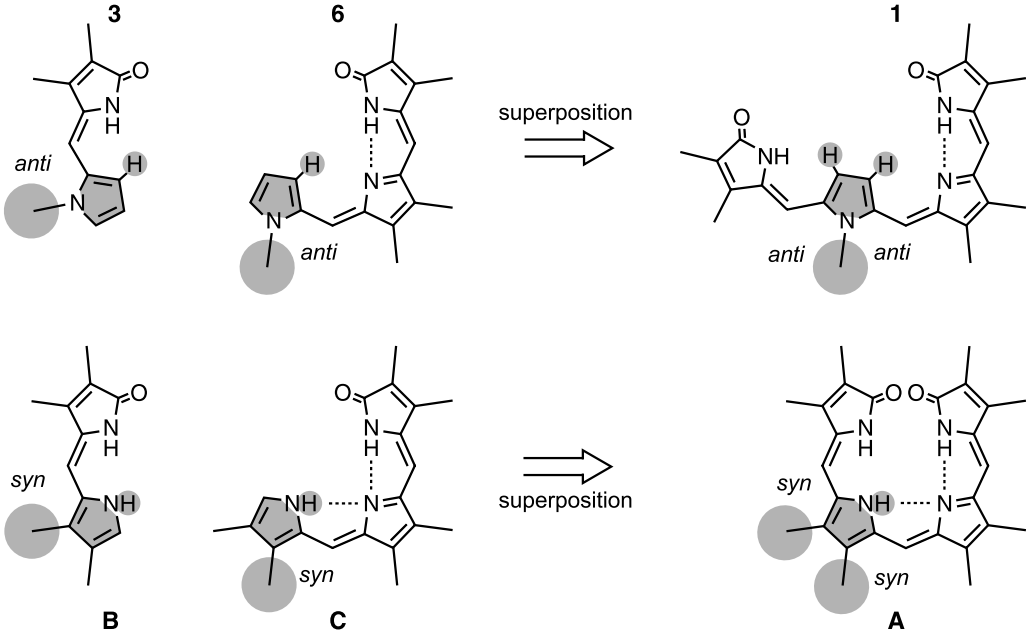


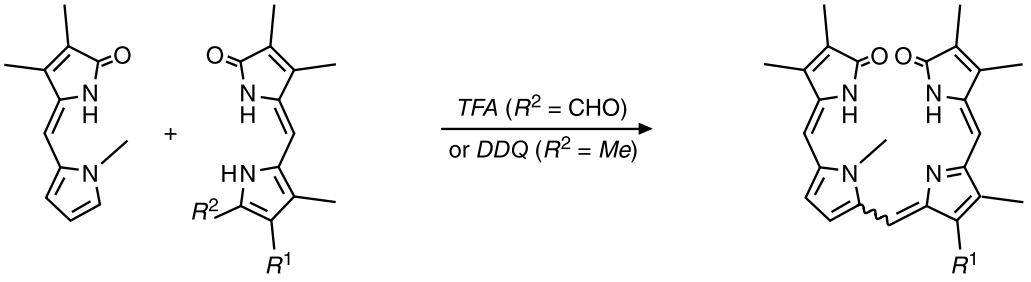
Scheme 1

whereas hydrogens should be located near the center regardless whether they are bound to nitrogen or β-carbon. Furthermore, *N*-methylation will interupt the NH$\cdots$N hydrogen bonding network of the entire chromophore thereby preventing tautomerism of the dipyrrin substructure. Consequently, bond rotation should take place on both sites of the *N*-methylated pyrrole leading to a novel (*anti–anti*)-conformer in biliverdins. In this paper we report on the influence of the 23*N*-methyl group on the chromophore geometry of biliverdins unsubstituted at the positions 8, 12, and 13.

Results and Discussions

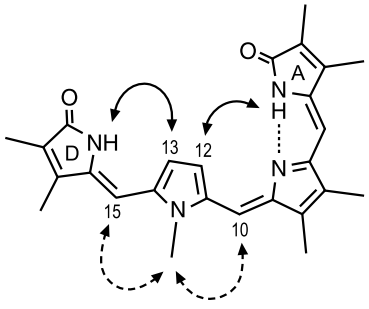
Biliverdins **1** and **2** both *N*-methylated at position 23 were synthesized by different coupling procedures from their pyrromethenone precursors (Scheme 2). Compound **1** was prepared under acid catalysis from 11*N*-methyldipyrrinone **3** and 9-formyldipyrrinone **4** following the procedure of *Falk* and *Thirring* [10], whereas compound **2** was synthesized by the oxidative coupling of **3** and 9-methyldipyrrinone **5** with two equivalents of *DDQ* according to the procedure of *Falk* and *Schlederer* [11]. With regard to the configuration of the double bond formed within the dipyrrin moiety biliverdin **1** was found to be the (*Z*)-diastereomer exclusively. However, biliverdin **2** was composed of both the (*Z*)- and the (*E*)-diastereomer **2a** and **2b** in a ratio of 1.00:0.45. Unfortunately, the diastereomers could not be separated by chromatographic methods such as *TLC* or *HPLC* even though their interconversion at room temperature can be excluded because of the absence of exchange peaks in their 2D-N*O*ESY spectra.

Assignments of the chromophore geometries were achieved by H,H-N*O*ESY measurements using *DMSO*-d_6 as the solvent to distinguish the hydrogen-bridged



		R^1	R^2	
3	**4**	*Me*	CHO	**1**
3	**5**	H	*Me*	**2a, 2b**

Scheme 2



1

Scheme 3

NH of lactam-A from that of the non hydrogen-bridged one of lactam-D. As expected, the stereochemistry of **1** corresponds to our assumption that the *N*-methylpyrrole subunits of dipyrrinone **3** and tripyrrinone **6** could simply be superimposed. In principle the (4*Z*,9*Z*,15*Z*,5*syn*,10*anti*,14*anti*)-stereochemistry could be deduced from two cross-peaks stemming from the lactam hydrogens and the β-hydrogens of the *N*-methylated pyrrole [NH-(lactam-A) $\leftrightarrow$ H-C12 and NH-(lactam-D) $\leftrightarrow$ H-C13] (Scheme 3). In addition, the double-*anti*-orientation of the *N*-methylpyrrole is corroborated by another pair of cross-peaks interconnecting the signals of the *N*-methyl group and the methine hydrogens at position 10 and 15.

Differences in stereochemistry of **2a** and **2b** could be assigned by finding opposite configurations of their dipyrrin subunits, namely (9*Z*) for **2a** and (9*E*) for **2b** (Fig. 1). All other constraints in geometry are similar for both of them: (4*Z*,5*syn*) and (15*Z*,14*anti*) within their dipyrrinone fragments and (10*anti*) for their dipyrrin moieties. Consequently, the chromophore geometry of **2a** corresponds with that of **1**, whereas **2b** represents the first stable (9*E*)-configurated biliverdin, whose configuration is neither fixed by covalent bridging of adjacent rings [12, 13] nor compelled by hydrogen bonds to an imidazole conjugated to the chromophore [14].

In comparison to **1**, the occurrence of **2b** is due to the absence of the methyl group at position 8 minimizing sterical hindrance with respect to the adjacent

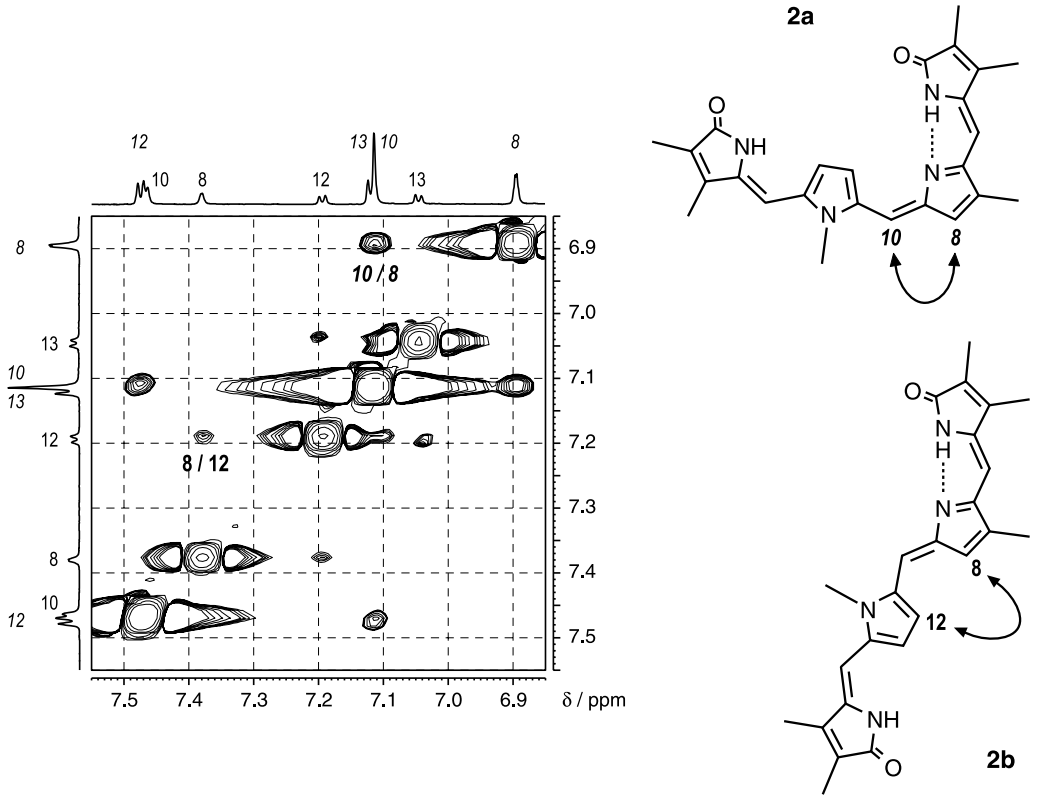
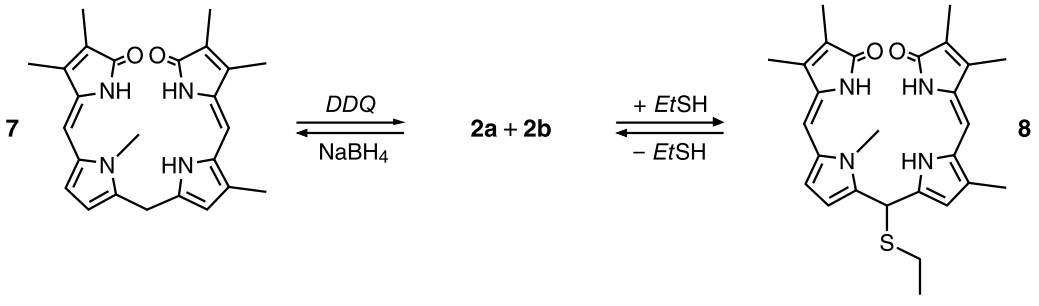


Fig. 1. Cross-peaks of geometrical relevance in the 2D-N*O*ESY spectrum of **2a**/**2b** in *DMSO*-d_6 (left) pointing to the differences in stereochemistry as shown by the double-headed arrows in the structural formulas (right); NMR-signals are numbered (italics: **2a**, regular: **2b**) in accordance with the position numbers of the corresponding hydrogens

β-hydrogen at position 12. Moreover, the availability of **2b** also results from the synthesis method used for its preparation when intermediate **7** is oxidized after its formation without isolation (Scheme 4). Assuming that transition state conformations during the second oxidation step resemble the configurations of the oxidation products, we prepared **7** by reduction of **2a**/**2b** with $NaBH_4$ and reoxidized it with *DDQ* in *THF* after its isolation. Again a mixture of **2a** and **2b** at a ratio of about 2:1 was isolated.



Scheme 4

Finally, we verified the capability of adding nucleophiles to the (9*E*)-configurated azafulvene element of **2b**. Additions of this type are well documented for (9*Z*)-configured bilindiones in terms of nucleophilicity, solvent, and temperature [15–18]. Using ethylmercaptan as the nucleophile and a mixture of **2a** and **2b** as the substrate the addition could be achieved quantitatively at 5°C in chloroform yielding the crystalline adduct **8**. Thus, addition to the (9*E*)-configured azafulvene must have taken place because thermal interconversion of **2a** and **2b** can be excluded by the absence of exchange peaks in their 2D-N*O*ESY spectra. In the crystalline state thiol adduct **8** is of remarkable stability withstanding thermal decomposition up to 100°C. To our knowledge **8** is the only representative among 10-sulfur-bound thiol adducts of biliverdins, which could be isolated up to now. We suppose that the stability of **8** is primarily restricted to unfavourable stereoelectronic requirements for thiol-elimination in the crystalline state, because elimination is easily initiated even at room temperature when **8** is dissolved in a mixture of chloroform and methanol.

Experimental

All chemicals were reagent grade. *THF* was distilled from sodium benzophenone ketyl. Flash column chromatography was performed on silica gel 60 (Merck, 0.063–0.200 mm) or Al_2O_3 90 (Merck, 0.063–0.200 mm). NMR spectra were recorded on a Brucker Avance DRX-500 spectrometer. Assignments of ^1H and ^{13}C NMR signals were achieved using N*O*ESY and HSQC experiments under standard instrument parameters. IR and UV-Vis spectra were recorded using a Bruker Tensor 27 and a Varian Cary 100 spectrometer. MS detection was performed using a quadrupole system Hewlett Packard 5989B and a pneumatically assisted electrospray ionisation interface Hewlett Packard 59987A. Dipyrrinones **3**, **4**, and **5** were prepared according to Ref. [2].

(4Z,9Z,15Z)-2,3,7,8,17,18,23-Heptamethylbilin-1,19-(21H,23H,24H)-dione (**1**, $C_{26}H_{28}N_4O_2$)

A solution of 14.6 mg of **3** (72 μmol) and 17.0 mg of **4** (70 μmol) in 1 cm^3 of *TFA* was stirred for 72 h at room temperature. After adding 5 cm^3 of CH_2Cl_2, 4 cm^3 of methanol, and 30 cm^3 of H_2O the mixture was extracted with CH_2Cl_2. All extracts were combined and washed with saturated aqueous $NaHCO_3$. After evaporation of the solvent the residue was subjected to column chromatography (first Al_2O_3, $CHCl_3$ and then silica gel, $CHCl_3$:*Me*OH = 100:1) to afford 7.0 mg of **1** (23%) as a blue solid. Mp >300°C; ^1H NMR (500 MHz, *DMSO*-d_6, 50°C): $\delta = 10.55$ (s, H-N21), 9.72 (s, H-N24), 7.43 (d, $J = 4.5$ Hz, H-C12), 7.09 (d, $J = 4.5$ Hz, H-C13), 6.99 (s, H-C10), 6.13 (s, H-C15), 6.08 (s, H-C5), 3.81 (s, CH_3-N23), 2.16 (s, CH_3-C8), 2.13 (s, CH_3-C17), 2.11 (s, CH_3-C3), 2.04 (s, CH_3-C7), 1.86 (s, CH_3-C2), 1.82 (s, CH_3-C18) ppm; N*O*ESY (500 MHz, *DMSO*-d_6, 50°C): CH_3-C2 ↔ CH_3-C3 ↔ H-C5 ↔ CH_3-C7 ↔ CH_3-C8 ↔ H-C10 ↔ CH_3-N23 ↔ H-C15 ↔ CH_3-C17 ↔ CH_3-C18, H-N21 ↔ H-C12 ↔ H-C13 ↔ H-N24; IR (KBr): $\bar{\nu} = 3227$, 2916, 1698, 1666, 1590, 1260, 1095 cm^{-1}; UV-Vis ($CHCl_3$): λ_{max} (ε) = 587 (16800), 370 (15900) nm ($mol^{-1}\,dm^3\,cm^{-1}$); UV-Vis (*DMSO*): λ_{max} (ε) = 611 (14000), 375 (13200) nm ($mol^{-1}\,dm^3\,cm^{-1}$); MS (ESIp): $m/z = 429$ [M + H^+].

(4Z,9Z,15Z)- and (4Z,9E,15Z)-2,3,7,17,18,23-Hexamethylbilin-1,19-(21H,23H,24H)-dione (**2a** and **2b**, $C_{25}H_{26}N_4O_2$)

A solution of 17.5 mg of **3** (86 μmol) and 18.7 mg of **5** (86 μmol) in 6 cm^3 of absolute *THF* was cooled to approximately −50°C. Then a solution of 40.5 mg of *DDQ* (180 μmol) in 10 cm^3 of absolute *THF* was added dropwise within 1 h. Under stirring the reaction mixture was allowed to warm up to 0°C and

was then poured into a cold mixture of 20 cm^3 of $CHCl_3$ and 30 cm^3 of aqueous triethylamine (1%). After extracting with $CHCl_3$, the combined extracts were washed with aqueous *L*-ascorbic acid (1%), H_2O, and dried (Na_2SO_4). After evaporation of the solvent the residue was subjected to column chromatography (Al_2O_3, $CHCl_3$:*Me*OH = 20:1) to afford 30.0 mg of **2a** and **2b** (84%) in a ratio of 1.00:0.45 as a blue solid. ^{1}H NMR (500 MHz, *DMSO*-d_6, 45°C): **2a**: $\delta = 10.58$ (s, H-N21), 9.73 (s, H-N24), 7.47 (d, $J = 4.4$ Hz, H-C12), 7.12 (d, $J = 4.4$ Hz, H-C13), 7.11 (s, H-C10), 6.89 (quartettoid, $J = 1.2$ Hz, H-C8), 6.13 (s, H-C15), 6.11 (s, H-C5), 3.79 (s, CH_3-N23), 2.17 (d, $J = 1.2$ Hz, CH_3-C7), 2.13 (s, CH_3-C3, CH_3-C17), 1.87, 1.82 (2s, CH_3-C2, CH_3-C18) ppm; **2b**: $\delta = 10.53$ (s, H-N21), 9.78 (s, H-N24), 7.46 (s, H-C10), 7.38 (quartettoid, $J = 1.2$ Hz, H-C8), 7.19 (d, $J = 4.4$ Hz, H-C12), 7.05 (d, $J = 4.4$ Hz, H-C13), 6.13 (s, H-C15), 6.07 (s, H-C5), 3.80 (s, CH_3-N23), 2.21 (d, $J = 1.2$ Hz, CH_3-C7), 2.13 (s, CH_3-C17), 2.11 (s, CH_3-C3), 1.85, 1.82 (2s, CH_3-C2, CH_3-C18) ppm; N*O*ESY (500 MHz, *DMSO*-d_6, 45°C): **2a**: (CH_3-C2, CH_3-C18) ↔ (CH_3-C3, CH_3-C17), CH_3-C3 ↔ H-C5 ↔ CH_3-C7 ↔ H-C8 ↔ H-C10 ↔ CH_3-N23 ↔ H-C15 ↔ CH_3-C17, H-N21 ↔ H-C12 ↔ H-C13 ↔ H-N24; **2b**: (CH_3-C2, CH_3-C18) ↔ (CH_3-C3, CH_3-C17), CH_3-C3 ↔ H-C5 ↔ CH_3-C7 ↔ H-C8 ↔ H-C12 ↔ H-C13 ↔ H-N24, H-C10 ↔ CH_3-N23 ↔ H-C15 ↔ CH_3-C17; IR (KBr): $\bar{\nu} = 3235$, 2916, 1684, 1594, 1340, 1286, 1131 cm^{-1}; UV-Vis ($CHCl_3$): λ_{max} (ε) = 570 (48200), 369 (18900) nm (mol^{-1} dm^3 cm^{-1}); UV-Vis (*DMSO*): λ_{max} (ε) = 608 (46600), 374 (28800) nm (mol^{-1} dm^3 cm^{-1}); MS (ESIp): $m/z = 415$ [M + H$^+$].

(4Z,15Z)-2,3,7,17,18,23-Hexamethylbiladien-ac-1,19(21H,24H)-dion (**7**, $C_{25}H_{28}N_4O_2$)

A mixture of 2.6 mg of **2a** and **2b** (6.3 μmol) was dissolved in 1 cm^3 of a 1:1 mixture of $CHCl_3$ and methanol and treated with 0.35 mg of $NaBH_4$ (9.3 μmol) under Ar. After 2 h the reaction mixture was diluted with 2 cm^3 of $CHCl_3$ and washed twice with H_2O. This procedure has to be done rapidly to avoid reoxidation. The organic layer was dried (Na_2SO_4) and the solvent was removed under vacuum. The residue was subjected to column chromatography (Al_2O_3, first $CHCl_3$ and then $CHCl_3$:*Me*OH = 10:1) to afford 2.3 mg of **7** (87%) as a yellow solid. Mp >300°C; ^{1}H NMR (500 MHz, *DMSO*-d_6, 30°C): $\delta = 10.52$ (s, H-N22), 9.78 (s, H-N21), 9.40 (s, H-N24), 6.68 (d, $J = 3.9$ Hz, H-C13), 6.02 (s, H-C15), 5.93 (s, H-C5), 5.89 (d, $J = 3.9$ Hz, H-C12), 5.63 (s, H-C8), 3.92 (s, H_2-C10), 3.52 (s, CH_3-N23), 2.06 (s, CH_3-C3, CH_3-C7, CH_3-C17), 1.75 (s, CH_3-C2, CH_3-C18) ppm; ^{13}C NMR (125 MHz, *DMSO*-d_6, 30°C): $\delta = 171.9$, 171.7 (C1, C19), 141.2 (C4, C16), 133.0, 132.9, 132.8 (C9, C11, C17 or C18), 129.0, 123.7 (C2, C3), 127.4 (C14), 124.8 (C17 or C18), 122.9, 122.7 (C6, C7), 110.1 (C13), 109.4 (C8), 108.4 (C12), 97.3 (C5), 97.2 (C15), 30.0 (C-N23), 25.1 (C10), 10.9, 9.3 (C-C3, C-C7, C-C17) 8.0 (C-C2, C-C18) ppm; IR (KBr): $\bar{\nu} = 3333$, 2917, 1666, 1638 cm^{-1}; UV-Vis (*Me*OH): λ_{max} (ε) = 411 (32200) 237 (10400) nm (mol^{-1} dm^3 cm^{-1}); MS (ESIp): $m/z = 417$ [M + H$^+$].

rac-(4Z,15Z)-10-Ethylthio-2,3,7,17,18,23-Hexamethylbiladien-ac-1,19(21H,24H)-dion (**8**, $C_{27}H_{32}N_4O_2S$)

A mixture of 3.8 mg of **2a** and **2b** (9.2 μmol) was dissolved in 2 cm^3 of $CHCl_3$ and treated with 1 mm^3 of ethanthiole (13.5 μmol). The reaction vessel was closed properly and stored at 5°C over night. After about 20 min the blue solution turned yellow and after a few hours the product started to crystallize. Removing the solvent under vacuum afforded 4.4 mg of **8** (100%) as greenish crystals. Mp 105°C (dec.); ^{1}H NMR (500 MHz, *DMSO*-d_6, 25°C): $\delta = 10.57$ (s, H-N22), 9.91 (s, H-N21), 9.49 (s, H-N24), 6.69 (d, $J = 4.0$ Hz, H-C13), 6.08 (d, $J = 4.0$ Hz, H-C12), 6.04 (s, H-C15), 6.02 (d, $J = 1.5$ Hz, H-C8), 5.94 (s, H-C5), 5.33 (s, H-C10), 3.65 (s, CH_3-N23), 2.49, 2.45 (2dq, $J_{AB} = 12.8$ Hz, $J_{AX} = J_{BX} = 7.4$ Hz, C(H$_x$)$_3$-C$H_A H_B$-S), 2.11 (s, CH_3-C7), 2.08, 2.07 (s, CH_3-C3, CH_3-C17), 1.78 (s, CH_3-C2, CH_3-C18), 1.52 (t, $J_{AX} = J_{BX} = 7.4$ Hz, C(H_x)$_3$-C$H_A H_B$-S) ppm; ^{13}C NMR (125 MHz, *DMSO*-d_6, 25°C): $\delta = 172.6$, 172.5 (C1, C19), 142.0, 141.9, 134.4, 134.4, 133.3, 130.3, 128.9, 125.8, 124.7, 123.9, 123.4 (C2, C3, C4, C6, C7, C9, C11, C14, C16, C17, C18), 111.2 (C8), 110.7 (C13),

110.2 (C12), 97.8 (C5), 97.5 (C15), 38.9 (C10), 30.8 (CH_3-N23), 25.9 (CH_2S), 14.8 (C-CH_2S), 11.7 (C-C7), 10.0 (C-C3, C-C17), 8.7 (C-C2, C-C18) ppm; IR (KBr): $\bar{\nu} = 3344$, 2920, 1681, 1660, 1474, 1170 cm^{-1}; UV-Vis (*DMSO*): λ_{max} (ε) = 413 (34600) nm ($mol^{-1}\,dm^3\,cm^{-1}$); MS (ESIp): $m/z = 477$ $[M + H^+]$.

Acknowledgements

M. Hölzl thanks the Johannes Kepler University Linz for a research fellowship.

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