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The Dissociation and Tautomerization Equilibria of Hypericin: Alkyl Protected Hydroxyl Derivatives

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Summary. 3-Benzyl-, 3,4-dibenzyl-, 3,4-dibenzyl-1,6,8,13-tetramethyl-, and 1,6,8,13-tetramethylhypericin were synthesized by alkylation and dealkylation procedures starting from hypericin. The pK_a value correlation of these derivatives allowed the unequivocal assignment of the protonation and deprotonation pK_a values of hypericin. Thus, for hypericin the pK_a of about −6 was assigned to the C=O groups, that of about 2 to the deprotonation of one hydroxyl group in the *bay*-positions 3/4, and that of about 9 was found to be characteristic of the *bay-peri*-diphenolate ion. None of the changes in the spectra characteristic of changes in the tautomeric equilibria could be found for these derivatives. Thus, it was concluded that the undisturbed peripheral hydroxyl groups of hypericin have to be present to allow for tautomeric changes.

Keywords. Deprotonation; pK_a values; Hypericin; Alkylhypericines; Tautomerism.

Die Dissoziations- und Tautomeriegleichgewichte von Hypericin: Alkylgeschützte Hydroxyderivate

Zusammenfassung. Die 3-Benzyl-, 3,4-Dibenzyl-, 3,4-Dibenzyl-1,6,8,13-tetramethyl- und 1,6,8,13-Tetramethylhypericinderivate wurden mit Hilfe von Alkylierungs- und Dealkylierungsverfahren synthetisiert. Die pK_a-Werte dieser Derivate erlaubten eine zweifelsfreie Zuordnung der Protonierungs- und Deprotonierungsschritte für Hypericin. Demzufolge ist der pK_a Wert von *ca.* −6 für die Protonierung der Carbonylgruppe, jener von *ca.* 2 für die Deprotonierung einer *bay*-Hydroxylgruppe und jener bei etwa 9 für das *bay-peri*-Diphenolation charakteristisch. Keine der charakteristischen spektroskopischen Änderungen für einen Wechsel im Tautomeriegleichgewicht von Hypericin konnte für diese Derivate belegt werden. Dementsprechend wurde geschlossen, daß intakte periphere Hydroxylgruppen im Hypericin die Voraussetzung für tautomere Änderungen bilden.

Introduction

Hypericin (**1**) occurs mostly in *Hypericum* species [1] and is valued for a variety of physiological effects [1, 2]. Several of them obviously depend mainly upon its

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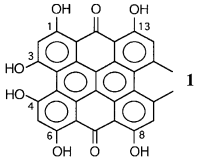
unique acid/base properties [3]. Moreover, **1** is prone to be involved in a tautomeric equilibrium involving the various hydroxyl and oxo groups of the molecule.



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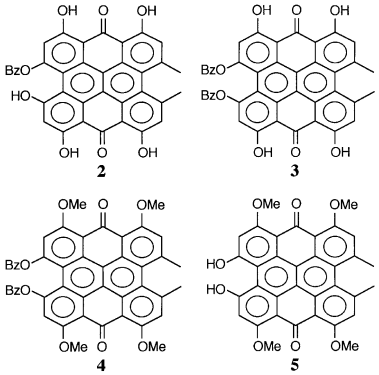
Studies to derive pK_a values of **1** by means of ^{1}H NMR, UV/Vis, and mass spectroscopy as well as electrophoresis, especially with respect to its physiologically meaningful deprotonation steps [4, 5, 6], have been undertaken and have provided values of about 1.8 and 9.2 for the *bay* – (positions 3, 4) and *peri*-hydroxyl (positions 1, 6, 8, 13) groups in aqueous solution. However, the positions of deprotonation have been evaluated only by indirect means, and an unequivocal proof is still lacking. Much the same situation is encountered with the tautomerization equilibrium which has been recently studied in some detail [7, 8].

One possibility to clarify this situation would be the study of hypericin derivatives selectively protected at the *bay*- and *peri*-positions, thus permitting ionization only at certain positions and, moreover, concomitantly freezing certain tautomeric states. This approach will be pursued in the present communication.

Results and Discussion

To provide some insight in the ionization selectivity of hypericin (**1**), the most important model systems would be those blocked in positions 3, $3+4$, and $1+6+8+13$. Correlation of the deprotonation pK_a values for such derivatives would allow to pinpoint the position of dissociation of **1**. At the same time, such a derivative would allow to freeze the tautomeric equilibrium at a certain stage, thus providing a means to isolate certain points of the complex equilibrium system. For these reasons, the model compounds **2** (with position 3 blocked by means of a benzyl group), **3** (with both *bay*-hydroxyl groups blocked by benzyl residues), and **5** (with the *peri*-hydroxyl groups blocked by methyl groups but free *bay*-region hydroxyl groups) were chosen. Compound **4** with all hydroxyl groups of hypericin alkylated could serve as a model for comparison.



Synthesis

The synthesis of the partially alkylated hypericin derivatives **2**, **3**, **4**, and **5** was first investigated by means of a biomimetic path by dimerizing alkylated emodin anthrone derivatives. However, it turned out that this approach was not rewarding due to partial dealkylations taking place under hypericin synthesis conditions. Therefore, direct alkylation procedures for **1** were developed. Thus, **1** could be regioselectively monobenzylated in position 3 by means of an about equimolar amount of benzyl bromide in presence of *Hünig*'s base to yield **2**, whereas the 3,4-dibenzylderivative **3** was regioselectively formed upon using an excess of these reagents. Permethylation of **3** to provide **4** was achieved by means of iodomethane in presence of potassium carbonate. Among the numerous methods available for debenzylation [9], the reaction of **4** with boron tribromide [10] worked best to yield the 3,4-unsubstituted *peri*-tetra-O-methyl hypericin derivative **5**.

Ionization

Starting for a correlation (cf. Fig. 1) with the completely alkylated derivative **4** only one pk_a step, the protonation of one carbonyl group at about -2, could be observed. Upon formal deblocking of the *bay*-region, which yields **5**, only one deprotonation step at about $pK_a = 3.5$ within the region up to 14 was discernible. This seemed to be plausible, since a second deprotonation step at the *bay*-region would produce a dianion with closely spaced negative charges – a species that would be extensively destabilized. Besides the deprotonation step, protonation of the carbonyl group appeared to be driven to a smaller basicity as compared to non alkylated derivatives. This is in agreement with the presence of electron donating alkyl groups in the vicinity of the carbonyl groups.

With the model system **3**, which is blocked at both *bay*-region hydroxyl groups, there was also one deprotonation step observable. However, it was considerably shifted into the region of $pK_a = 12$ which is characteristic of hydrogen bonded phenol dissociation. The protonation step of **3** demonstrated, of course, that dealkylation in the vicinity of the carbonyl group had the most profound consequences, placing this equilibrium at a pK_a values of about -6.

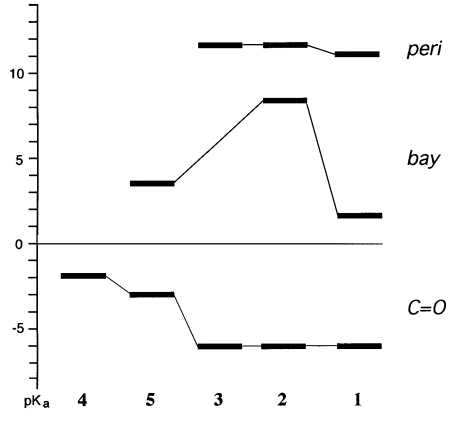


Fig. 1. pK_a correlations for protonation (C=O, aqueous H_2SO_4) and mono- and dideprotonation (aqueous 80% ethanol) at the *bay*- and *peri*-regions of **1**–**5**

Besides this unchanged protonation step, two deprotonation steps were observed in the mono-*bay*-hydroxyl blocked derivative **2**. The higher pK_a value corresponded to the deprotonation in the *peri*-region, whereas the lower one at a pK_a value of about 8 had to be attributed to the *bay*-region. This behavior is due to the destabilization of the formed phenolate ion by interaction with the closely spaced lone pair of the ether oxygen atom of the benzyloxy group. The enhanced acidity of this *bay*-hydroxyl group as compared to the *peri*-hydroxyl group is due to the delocalization inherent to its vinylogous carboxylic acid structure [4, 11].

Upon splitting off the last protecting group to formally yield hypericin (**1**) itself, stabilization of the *bay*-mono-deprotonated species was, in addition to the vinylogous carboxylic acid delocalization, dramatically enhanced by hydrogen bonding between the negative charge and the adjacent hydroxyl group [4, 5, 11]. The *peri*-deprotonation step was observed to be more or less uninfluenced by the last formal deprotection step. Thus, by correlation of the pK_a values of the selectively blocked derivatives **2–5**, the nature of the deprotonations steps of hypericin (**1**) could be unequivocally assigned as indicated in Fig. 1.

Tautomerism

Recently, certain changes in the UV/Vis and NMR spectra of **1** and its *bay*-phenolate, induced by their high concentrations in solvents like tetrahydrofuran or upon addition of protons or *Lewis* acids, have been found to be characteristic for the tautomerization of the normal 7,14-tautomer into the 1,6-tautomer [7, 8]. Investigation of the alkylated hypericin derivatives **2–5** did not reveal any of these changes, and therefore one could conclude that the formation of hypericin tautomers is obviously critically dependent upon an undisturbed hydroxyl groups periphery both in the *bay*- and the *peri*-regions. The derivatives **2**, **3**, and **5** were found to be in the 7,14-tautomer state by means of strategic NOE correlations.

Experimental

Melting points were taken by means of a Kofler hot stage microscope (Reichert, Vienna). ^1H, ^{13}C, IR, UV/Vis, and fluorescence spectra were recorded using Bruker DPX-200 and 500, Biorad-FT-IR-45, Perkin-Elmer IR-710B, and Hitachi-U-3210 and F-4010 instruments. Spectrophotometric titrations were carried out as described in Ref. [11] using 80% aqueous ethanol solutions and tetramethylammonium hydroxide or trifluoroacetic acid as titrants for pK_a values above 0, and a series of aqueous H_2SO_4 solutions with H_0 values ranging from 0 to -8 for pK_a values below 0. For fluorescence spectroscopy, 95% ethanol of *für die Fluoreszenzspektroskopie* grade (Merck), otherwise *p.a.* solvents were used. For the determination of the fluorescence quantum yields, Rhodamine B fluorescence ($\Phi_f = 0.69$; ethanol) was compared as the standard. Atmospheric pressure ionization mass spectrometry (cf. Ref. [6]) was performed in negative ion mode using a quadrupole instrument (HP 5989B, Hewlett Packard, Palo Alto, CA) equipped with an atmospheric pressure ionization interface (HP 59987A). Hypericin (**1**) was prepared by means of the recently described semi-synthetic procedure from emodin which was extracted from the commercially available bark *Cortex frangulae* [13].

3-Benzyloxy-1,4,6,8,13-pentahydroxy-phenanthro[1,10,9,8-opqra]perylene-7,14-dione (**2**; $C_{37}H_{22}O_8$)

A mixture of 30 mg **1** and 8 µl N-ethyl-N,N-diisopropyl amine in 2.5 ml dry CH_3CN was heated for 15 min. Then 30 µl benzyl bromide dissolved in 1 ml CH_3CN were added, and the reaction mixture

was refluxed for further 3 h, cooled to room temperature, and 1 ml 2 *N* HCL was added. After stirring for 10 min, the mixture was extracted with 300 ml ethyl acetate, washed twice with brine and then water, dried over Na_2SO_4, evaporated, and washed with petrol ether to remove unreacted benzyl bromide. Column chromatography (silica) of the crude product using first a mixture of $CHCl_3$/MeOH = 40/1 as eluent gave 13 mg (85% based upon reacted **1**) of the product. By elution with $CHCl_3$/MeOH = 1/1, 17 mg of unreacted hypericin were recovered.

M.p.: 240°C; ^{1}H NMR ($CDCl_3$, δ, 200 MHz): 14.41 (s, OH-1 or OH-6), 14.25 (s, OH-6 or OH-1), 13.81 (s, OH-8 or OH-13), 13.65 (s, OH-13 or OH-8), 9.21 (bs, OH-4), 7.4 (m, C_6H_5), 7.36 (s, ar-H-9, 12), 7.20 (s, ar-H-2 or ar-H-5), 6.95 (s, ar-H-5 or ar-H-2), 5.59 and 5.39 (AB-system, $J = 11$ Hz, OCH_2Ph), 2.73 (s, CH_3), 2.71 (s, CH_3) ppm; ^{1}H NOESY ($CDCl_3$): OH-1$\leftrightarrow$H-2$\leftrightarrow$$OCH_2Ph$, H-*ortho*-phenyl)$\leftrightarrow$$OCH_2Ph\leftrightarrow$H-2, CH_3-11$\leftrightarrow$$CH_3-10\leftrightarrow$H-9, CH_3-10$\leftrightarrow$$CH_3-11\leftrightarrow$H-12; ^{1}H NMR (*DMSO*-$d_6$, δ, 200 MHz): 14.08 (s, OH-1 or OH-6), 13.93 (s, OH-6 or OH-1), 13.58 (s, OH-8 or OH-13), 13.51 (s, OH-13 or OH-8), 12.02 (bs, OH-4), 7.4 (m, C_6H_5), 7.19 (s, ar-H-9, 12), 7.09 (s, ar-H-2 or ar-H-5), 6.73 (s, ar-H-5 or ar-H-2), 5.47 and 5.32 (AB-system, $J = 14$ Hz, OCH_2Ph), 2.37 (s, $2CH_3$) ppm; ^{1}H NMR (*THF*-d_8, δ, 500 MHz): 14.17 (s, OH-1 or OH-6), 14.07 (s, OH-6 or OH-1), 13.64 (s, Oh-8 or OH-13), 13.57 (s, OH-13 or OH-8), 10.7 (bs, OH-4), 7.5–7.2 (m, C_6H_5 + ar-H-9, 12), 7.17 (s, ar-H-2 or ar-H-5), 6.71 (s, ar-H-5 or ar-H-2), 5.49 and 5.44 (AB-system, $J = 11$ Hz, OCH_2Ph), 2.71 (s, $2CH_3$) ppm; ^{1}H NMR(*DMF*-d_7, δ, 200 MHz): 14.25 (s, OH-1 or OH-6), 13.12 (s, OH-6 or OH-1), 13.76 (s, OH-8 or OH-13), 13.68 (s, OH-13 or OH-8), 5.59 (s, OCH_2Ph), 2.7 (s, $2CH_3$, overlapped with *DMF* peak) ppm; ^{1}H NMR (*DMF*-d_7 + a drop of *TFA*, δ, 200 MHz): 14.25 (s, OH-1 or OH-6), 13.12 (s, OH-6 or OH-1), 13.75 (s, OH-8 or OH-13), 13.68 (s, OH-13 or OH-8), 12.62 (bs, OH-4), 5.59 (s, OCH_2Ph), 2.7 (s, $2CH_3$ overlapped with *DMF* peak); UV/Vis (ethanol, $3\cdot10^{-5}$ mol/l): λ_{max} (ε) = 609 (11033), 581 (13803), 540 (7407), 501 (5222), 439 (10837), 329 (16911) nm; UV/Vis (ethanol, $3\cdot10^{-5}$ mol/l, $pH \approx 2$): λ_{max} (ε) = 581 (32666), 540 (16418), 503 (5855), 452 (15359), 428 (11162), 320 (21625) nm; UV/Vis (ethanol, $3\cdot10^{-5}$ mol/l, $pH \approx 10$): λ_{max} (ε) = 610 (20611), 567 (13152), 491 (7977), 437 (14885), 330 (23975) nm; UV/Vis (ethanol, $3\cdot10^{-5}$ mol/l, $pH \approx 12$) λ_{max} (ε) = 616 (31900), 570 (16951), 529 (6574), 414 (14148), 353 (31648) nm; UV/Vis (CH_3OH, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 610 (11985), 579 (12149), 439 (9186), 327 (17626) nm; UV/Vis (CH_3OH, $1\cdot10^{-5}$ mol/1, $pH \approx 2$): λ_{max} (ε) = 579 (24566), 538 (12675), 502 (4375), 451 (12975), 428 (9616) nm; UV/Vis (*DMSO*, $1\cdot10^{-5}$ mol/1): λ_{max} (ε) = 585 (38064), 543 (19299), 507 (6721), 457 (19707), 434 (14457) nm; UV/Vis (*DMSO*, $1\cdot10^{-5}$ mol/l, $pH = 12$): λ_{max} (ε) = 625 (20940), 579 (11393), 419 (13420), 363 (27706) nm; UV/Vis (acetone, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 633 (3790), 581 (25387), 539 (13064), 504 (5862), 449 (14830), 329 (21120) nm; UV/Vis (acetone, $1\cdot10^{-5}$ mol/l, $pH \approx 2$): λ_{max} (ε) = 581 (40000), 539 (20362), 504 (7064), 452 (21064), 428 (14733), 329 (28929) nm; UV/Vis (acetonitrile, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 623 (4035), 579 (17927), 566 (15419), 540 (10467), 442 (12774), 324 (20943) nm; UV/Vis (acetonitrile, $1\cdot10^{-5}$ mol/l, $pH = 2$): λ_{max} (ε) = 579 (34083), 539 (18508), 503 (6966), 451 (20250), 429 (14483) nm; UV/Vis (pyridine, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 588 (36428), 545 (17778), 508 (6421), 456 (18507), 437 (15114), 334 (26428) nm; UV/Vis (*DMF*, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 624 (16306), 578 (9007), 501 (4201), 436 (11738), 417 (10910), 364 (21888) nm; UV/Vis (*DMF*, $1\cdot10^{-5}$ mol/l, $pH \approx 2$): λ_{max} (ε) = 585 (33746), 543 (16738), 507 (5500), 456 (16730), 432 (11900) nm; UV/Vis (*THF*, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 583 (39725), 541 (19572), 505 (6535), 453 (20153), 429 (14250), 320 (26677) nm; UV/Vis (ethyl acetate, $1\cdot10^{-5}$ mol/l) λ_{max} (ε) = 581 (41250), 539 (20354), 503 (6508), 451 (20830), 428 (14306), 319 (26870) nm; UV/Vis ($CHCl_3$, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 583 (34508), 542 (17798), 506 (5604), 454 (21032), 433 (14080), 318 (24879) nm; fluorescence (CH_3OH, $\lambda_{exc} = 550$ nm): λ_{em} (rel. intensity) = 584 (1), 630 (0.76) nm, $\Phi_f = 0.8$; fluorescence (ethanol, $\lambda_{exc} = 550$ nm): λ_{em} (rel. intensity) = 585 (1), 632 (0.37) nm, $\Phi_f = 0.4$; fluorescence (*DMSO*, $\lambda_{exc} = 550$ nm): λ_{em} (rel. intensity) = 595 (1), 641 (0.3) nm, $\Phi_f = 0.8$; fluorescence (*DMF*, $\lambda_{exc} = 550$ nm): λ_{em} (rel. intensity) = 594 (1), 641 (0.5) nm, $\Phi_f = 0.7$; fluorescence (pyridine, $\lambda_{exc} = 550$ nm): λ_{em} (rel. intensity) = 594 (1), 639 (0.6) nm, $\Phi_f = 0.5$; fluorescence (*THF*, $\lambda_{exc} = 550$ nm): λ_{em} (rel. intensity) = 587 (1), 635 (0.3) nm, $\Phi_f = 0.7$; fluorescence (ethyl acetate, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 589$ (1), 635 (0.3) nm, $\Phi_f = 0.7$; fluorescence ($CHCl_3$, $\lambda_{exc} = 550$ nm): λ_{em} (rel.

intensity) = 590 (1), 636 (0.3) nm, $\Phi_f = 0.8$; fluorescence (80% ethanol, $\lambda_{exc} = 550$ nm): λ_{em} (rel. intensity): **2**: 585 (1), 632) (2.7), $\mathbf{2}^-$: 647, $\mathbf{2}^{2-}$: 650 nm; IR (KBr): $\nu = 3413, 2927, 1603, 1548, 1471, 1413, 1386, 1325, 1283, 1263, 1237, 1220, 1193, 1115, 836, 801, 731, 673, 583\,cm^{-1}$; MS (e-spray ionization from *THF*/H_2O) *m/z* (%) = 593 (11, M-H^+), 503 (54), 339 (1), 325 (10), 310 (6), 117 (63), 111 (4), 103 (100), 101 (10), 89 (5), 85 (9), 73 (16); pK_a determinations: protonation: $\lambda_{\mathbf{1}} = 590$ nm, $\lambda_{2\cdot H^+} = 648$ nm, $\varepsilon_\lambda/\varepsilon_{\lambda\cdot H^+} = 0.83$; $pK_a(p) = -6.1 \pm 0.2$; deprotonation (80% ethanol): $\lambda_{\mathbf{2}} = 581$ nm, $\lambda_{2^-} = 610$ nm, $\varepsilon_\lambda/\varepsilon_{\lambda^-} = 1.71$; pK_a $(d_1) = 8.3 \pm 0.3$; $\lambda_{\mathbf{2}^-} = 615$ nm, $\varepsilon_\lambda/\varepsilon_{\lambda^{2-}} = 1.48$; pK_a $(d_2) = 11.7 \pm 0.1$.

3,4-Dibenzyloxy-1,6,8,13-tetrahydroxy-phenanthro[1,10,9,8-opqra]perylene-7,14-dione (**3**; $C_{44}H_{28}O_8$)

A mixture of 30 mg **1** and 30 µl N-ethyl-N,N-diisopropyl amine in 6 ml dry CH_2CN was heated for 15 min. Then 120 µl benzyl bromide in 2 ml CH_3CN were added, and the reaction mixture was refluxed for further 4 h; after cooling to room temperature, 3 ml of 2 *N* HCl were added. After stirring for 10 min, the mixture was extracted with 300 ml ethyl acetate, washed twice with brine and then water, dried over Na_2SO_4, evaporated, and washed with petrol ether to remove unreacted benzyl bromide. Column chromatography (silica) of the crude product using a mixture of $CHCl_3$/MeOH = 40/1 as eluent gave 18 mg (77%, based upon reacted **1**) of **3**. Elution with $CHCl_3$/MeOH = 1/1 afforded 13 mg unreacted hypericin.

M.p.: 249°C; ^{1}H NMR ($CDCl_3$, δ, 200 MHz): 14.31 (s, OH-1 + OH-6), 13.65 (s, OH-13 + OH-8), 7.36 (m, H-*meta* + *para* of $2C_6H_5$), 7.24 (s, ar-H-9, 12), 7.11 (m, H-*ortho* of $2C_6H_5$), 7.03 (s, ar-H-2,5), 5.11 and 54.87 (AB-system, $J = 11$ Hz, $2OCH_2Ph$), 2.54 (s, $2CH_3$) ppm; ^{1}H NOESY ($CDCl_3$, δ, 200 MHz): OH-1, 6 ↔ H-2,5 ↔ OCH_2Ph, H-2,5 ↔ OCH_2Ph ↔ H-*ortho* (of phenyl), OH-8,13 ↔ H-9,12 ↔ CH_3, H-2,5 ↔ OH-1,6 ↔ OH-8,13, H-9,12 ↔ OH-8,13 ↔ OH-1,6; ^{1}H NMR (*THF*-d_8, δ, 500 MHz): 14.17 (s, OH-1 + OH-6), 13.53 (s, OH-13 + OH-8) 7.32 (m, H-*meta* + *para* of $2C_6H_5$), 7.18 (m, ar-H-9,12 + H-*ortho* of $2C_6H_5$), 7.06 (s, ar-H-2,5), 5.22 and 5.02 (AB-system, $J = 12$ Hz, $2OCH_2Ph$), 2.50 (s, $2CH_3$) ppm; ^{13}C NMR (*THF*, δ, 50 MHz): 188.2 (C=O), 168.9 (C_{ar}), 165.3 (C_{ar}), 164.0 (C_{ar}), 146.3 (C_{ar}), 129.3 (C_{ar}), 129.1 (CH_{ar}), 128.8 (C_{ar}), 128.5 (CH_{ar}), 127.5 (CH_{ar}), 122.6 (C_{ar}), 120.5 (CH_{ar}), 109.2 (C_{ar}), 105.1 (C_{ar}), 101.1 (CH_{ar}), 71.3 (OCH_2Ph), 24.1 (CH_3) ppm; UV/Vis (CH_3OH, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 578 (32636), 537 (17481), 502 (5954), 448 (18600), 424 (13618), 327 (17626) nm; UV/Vis (*DMSO*, $6\cdot10^{-6}$ mol/l): λ_{max} (ε) = 583 (40365), 542 (20031), 506 (5428), 453 (20492), 429 (13111) nm; UV/Vis (acetone, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 579 (42836), 538 (21663), 503 (7100), 449 (22100), 425 (15200), 331 (27600) nm; UV/Vis (acetonitrile, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 578 (41545), 537 (21763), 502 (7100), 448 (22145), 426 (15463), 319 (28809) nm; UV/Vis (*DMF*, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 615 (11454), 583 (30500), 541 (15027), 453 (20618), 333 (28290) nm; UV/Vis (*THF*, $4\cdot10^{-5}$ mol/l): λ_{max} (ε) = 582 (46695), 540 (23240), 505 (7419), 451 (23564), 427 (14857), 330 (29047) nm; UV/Vis (ethyl acetate, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 579 (48072), 538 (24109), 503 (7927), 448 (24472), 425 (16772), 319 (3300) nm; UV/Vis ($CHCl_3$, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 582 (42790), 540 (22036), 505 (7309), 450 (24927), 427 (16581), 321 (32254) nm; fluorescence (CH_3OH, $\lambda_{exc} = 550$ nm): λ_{em} (rel. intensity) = 584 (1), 629 (0.56) nm, $\Phi_f = 0.5$; fluorescence (*DMF*, $\lambda_{exc} = 550$ nm): λ_{em} (rel. intensity) = 590 (1), 635 (0.76) nm, $\Phi_f = 0.6$; fluorescence (acetonitrile, $\lambda_{exc} = 550$ nm): λ_{em} (rel. intensity) = 585 (1), 631 (0.3) nm, $\Phi_f = 0.6$; fluorescence (*THF*, $\lambda_{exc} = 550$ nm): λ_{em} (rel. intensity) = 587 (1), 635 (0.3) nm, $\Phi_f = 0.7$; fluorescence (ethyl acetate, $\lambda_{exc} = 550$ nm): λ_{em} (rel. intensity) = 585 (1), 631 (0.3) nm, $\Phi_f = 0.7$; fluorescence ($CHCl_3$, $\lambda_{exc} = 550$ nm): λ_{em} (rel. intensity) = 590 (1), 636 (0.3) nm, $\Phi_f = 0.8$; IR (KBr): $\nu = 3437$ (br), 3031, 2929, 2861 (w), 1729 (w), 1597, 1542, 1494, 1474, 1416, 1387, 1320, 1279, 1232, 1193, 1026, 1002, 943, 910, 801, 735, 697, 681, 630, 625 cm^{-1}; MS (e-spray ionization from NaOH/ethanol/H_2O): *m*/z (%) = 683 (5, M-H^+), 452 (3), 339 (22), 325 (85), 311 (100), 297 (13), 281 (13), 255 (20), 227 (22), 162 (7), 157 (15), 142 (12); pK_a determinations: protonation:

$\lambda_{\mathbf{3}} = 590$ nm, $\lambda_{\mathbf{3}\cdot H^+} = 650$ nm, $\varepsilon_\lambda/\varepsilon_{\lambda\cdot H^+} = 0.83$; $pK_a(p) = -6.0 \pm 0.2$; deprotonation (80% ethanol): $\lambda_{\mathbf{3}} = 581$ nm, $\lambda_{\mathbf{3}^-} = 640$ nm, $\varepsilon_\lambda/\varepsilon_{\lambda^-} = 1.71$; pK_a (d_1) $= 11.7 \pm 0.3$.

3,4-Dibenzyloxy-1,6,8,13-tetramethoxy-phenanthro[1,10,9,8-opqra]perylene-7,14-dione (**4**; $C_{48}H_{36}O_8$)

A mixture of 35 mg **3** (0.05 mmol), 72 mg CH_3J (0.5 mmol), and 2.0 g K_2CO_3 in 50 ml anhydrous acetone was heated at reflux for 12 h. The mixture was cooled and, after addition of H_2O, extracted with 2×100 ml $CHCl_3$, washed twice with brine and water, dried over Na_2SO_4, and evaporated. The orange-red residue was chromatographed with $CHCl_3/CH_3OH$ (40/1) on silica, yielding 30 mg (79%) **4**.

M.p.: not below 350°C; ^{1}H NMR ($CDCl_3$, δ, 200 MHz): 7.34–7.32 (m, *ortho* + *meta*-H of $2C_6H_5$), 7.19 (m, H-9,12 + *para*-H of $2C_6H_5$), 6.94 (s, H-2,5), 5.04 (AB-system, $J_{AB}\approx10$ Hz, $2OCH_2Ph$), 4.21 (s, $2OCH_3$), 4.18 (s, $2OCH_3$), 2.72 (s, $2CH_3$) ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 170.5 (C=O), 164.0 (C_{ar}), 150.5 (C_{ar}), 145.5 (C_{ar}), 138.7 (C_{ar}), 138.2 (C_{ar}), 137.9 (C_{ar}), 137.7 (C_{ar}), 137.4 (C_{ar}), 136.2 (C_{ar}), 135.4 (C_{ar}), 132.1 (C_{ar}), 124.0 (C_{ar}), 121.3 (C_{ar}), 106.3 (C_{ar}), 79.9 ($2OCH_2$), 66.3 ($2OCH_3$), 66.2 ($2OCH_3$), 32.9 ($2CH_3$) ppm; IR (KBr): $\nu = 2931$, 2845, 1723, 1639, 1587, 1593, 1461, 1407, 1371, 1322, 1284, 1235, 1225, 1004, 905, 846, 821, 737, 697 cm^{-1}; UV/Vis (ethanol, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 533 (26690), 465 (9710), 424 (25090), 368 (9710) nm, UV/Vis (CH_3OH, $8\cdot10^{-6}$ mol/l): λ_{max} (ε) = 535 (23370), 467 (8750), 426 (22020), 369 (9350) nm; UV/Vis ($CHCl_3$, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 582 (38030), 575 (32000), 526 (29430), 467 (12590), 423 (31300), 368 (12400) nm; UV/Vis (*THF*, $1\cdot10^{-5}$ mol/l): λ_{max} (ε) = 513 (38300), 453 (17900), 414 (42340), 362 (19190) nm; fluorescence (ethanol, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 560$ nm, $\Phi_f = 0.76$; fluorescence (CH_3OH, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 562$, $\Phi_f = 0.71$; fluorescence ($CHCl_3$, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 589$ nm, $\Phi_f = 0.76$; fluorescence (*THF*, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 589$ nm, $\Phi_f = 0.36$; protonation: pK_a (aqueous H_2SO_4) $\approx -2 \pm 0.4$, $\lambda_{\mathbf{4}\cdot H^+} = 653$ nm.

3,4-Dihydroxy-1,6,8,13-tetramethoxy-phenanthro[1,10,9,8-opqra]perylene-7,14-dione (**5**; $C_{34}H_{24}O_8$)

A solution of 37 mg **4** (0.05 mmol) in 10 ml dry CH_2Cl_2 was cooled to −100°C using liqu. N_2. To this solution, a solution of 0.18 ml BBr_3 in 10 ml CH_2Cl_2 was added dropwise during 30 min under stirring at −100°C. Then the cooling both was removed, and the temperature was allowed to rise slowly during 1.5 h to 5°C. 5 ml CH_2Cl_2 + 8 ml *N* HCl were added at once, and the organic phase was extracted with 2×100 ml 0.5 *N* Na_2CO_3. Upon acidification, the precipitate was purified on a Sephadex LH-20 column with CH_3OH as eluent to yield 7 mg **5** (25%).

M.p.: 185°C; ^{1}H NMR (*DMSO*-d_6, δ, 200 MHz): 17.41 (s, OH-4), 7.41 (s, H-9,12), 6.72 (s, H-2,5), 4.01 (s, OCH_3-8,13), 3.92 (s, OCH_3-1,6), 2.60 ($2CH_3$) ppm; ^{1}H NOESY (*DMSO*-d_6, 200 MHz): OCH_3-8,13 ↔ H, 9,12 ↔ CH_3-10,11, OCH_3-1,6 ↔ H-2,5 ↔ OH-3/4; ^{1}H NMR (CD_3OD, δ, 200 MHz): 7.49 (s, H-9,12), 6.99 (s, H-2,5), 4.14 (s, OCH_3-8,13), 4.10 (s, OCH_3-1,6), 2.74 (2, $2CH_3$) ppm; ^{13}C NMR (*DMSO*-d_6, δ, 50 MHz): 180.8 (C=O), 169.6, 161.9 (C_{ar}), 157.6 (C_{ar}), 139.4 (C_{ar}), 139.3 (C_{ar}), 139.2 (C_{ar}), 128.8 (C_{ar}), 125.9 (C_{ar}), 123.1 (C_{ar}), 121.4 (C_{ar}), 116.9 (C_{ar}), 116.7 (C_{ar}), 103.2 (C_{ar}), 56.6 (OCH_3), 55.8 (OCH_3), 23.18 (CH_3) ppm; UV/Vis (ethanol 80%, $1\cdot10^{-6}$ mol/l, *pH*≈6): λ_{max} (ε) = 558 (35780), 494 (20000), 456 (34460), 391 (21680), 390 (22920), 386 (21620), 373 (23080), 358 (21580) nm; UV/Vis (CH_3OH, $5\cdot10^{-6}$ mol/l): λ_{max} (ε) = 554 (33620), 493 (20520), 450 (33820), 390 (23880), 384 (24080) nm; UV/Vis (acetone, $5\cdot10^{-6}$ mol/l): λ_{max} (ε) = 561 (23800), 480 (16760), 452 (31140), 400 (20200) nm; UV/Vis (acetonitrile, $5\cdot10^{-6}$ mol/l): λ_{max} (ε) = 552 (21240), 482 (13720), 450 (24840), λ_{max} (ε) = 399 (16320), 389 (18020), 387 (16780) nm; UV/Vis (*DMF*, $5\cdot10^{-6}$ mol/l): λ_{max} (ε) = 559 (22100), 489 (16600), 456 (28400), 401 (19180), 389 (22720), 385 (21160) nm; UV/Vis (*DMSO*, $5\cdot10^{-6}$ mol/l): λ_{max} (ε) = 561 (36500), 488 (24980), 456 (44440), 403 (28860) nm; UV/Vis (*THF*, only very faintly soluble): λ_{max} (ε) = 560 (8), 496 (5), 463 (10), 407 (6), 390 (9), 385 (7) nm; UV/Vis ($CHCl_3$, only very faintly soluble): λ_{max} (ε): = 590

(16), 452 (10), 495 (16), 452 (10), 395 (15), 365 (10) nm; fluorescence (ethanol 80%, $\lambda_{exc} =$ 550 nm): $\lambda_{em} = 610$ nm, $\Phi_f = 0.19$; fluorescence (CH_3OH, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 607$ nm, $\Phi_f = 0.21$; fluorescence (acetone, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 619$ nm, $\Phi_f = 0.17$; fluorescence (acetonitrile, $\lambda_{exc} =$ 550 nm): $\lambda_{em} = 618$ nm, $\Phi_f = 0.14$; fluorescence (*DMF*, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 619$ nm, $\Phi_f = 0.19$; fluorescence (*DMSO*, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 619$ nm, $\Phi_f = 0.24$; IR (KBr): $\nu = 3436$, 2924, 1727 (w), 1607, 1548, 1514, 1471, 1403, 1261, 1115, 999, 840 cm^{-1}; MS (e-spray from CH_3OH): $m/z = 559$ ($M\text{-}H^+$), 509, 483, 353, 281, 255 (100%), 227, 189, 1434, 129; protonation (aqu. H_2SO_4): $pK_4 = -3 \pm 0.3$, $\lambda_{\mathbf{5}\cdot H^+} = 655$ nm; deprotonation (80% ethanol): $pK_a = 3.5 \pm 0.2$, $\lambda_{\mathbf{5}^-} = 558$ nm, $\lambda_{\mathbf{5}} =$ 595 nm, $\varepsilon_{\mathbf{5}^-}/\varepsilon_{\mathbf{5}} \approx 1$.

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