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On synthesis and properties of hypericin-porphyrin hybrids

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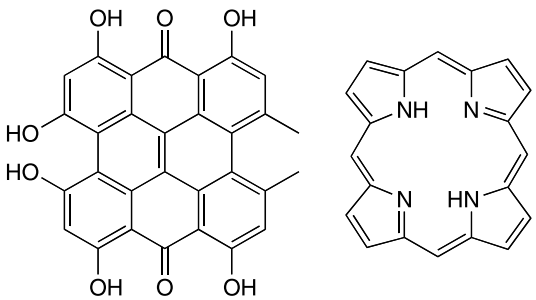
Abstract Two diastereomeric tetraphenylporphyrinyl-ω-hypericinyl-ethylenes were prepared and their properties investigated. The (*Z*)-diastereomer displayed an even higher photosensitization of singlet oxygen and/or reactive oxygen species than hypericin, whereas the (*E*)-configured derivative showed a somewhat weaker effect. Accordingly, hybridization of hypericin and porphyrin chromophores seems to be a promising target for the development of novel sensitizers for photodynamic therapy.

Keywords Hypericin; Porphyrin; Singlet oxygen; Reactive oxidative species; Absorption.

Introduction

Hypericin (**1**) on the one side and derivatives of porphyrin (**2**), among them the celebrated Photofrin®, on the other side, are on the forefront of sensitizers used in photodynamic therapy [1–3]. In both areas numerous investigations have been dedicated to improve these photosensitizers by appropriate derivatization to yield higher-generation agents. Among such studies hybridization of different chromophores or pharmacophores is a promising avenue [4]. Thus, we set out to explore the hybridization of hypericin with porphyrin to unite the intense long-wavelength absorption of hypericin with the very weakly absorbing, but highly tumor-targeting properties of porphyrins [5]. With respect to the hypericin moiety the methyl groups have proven hitherto to be ideally suited to serve as ''anchors'' for substitution because the photophysical properties of the hypericin chromophore remain more or less untouched by such derivatizations [2]. With respect to the porphyrin moiety we chose a most easily accessible precursor system, namely a *meso*-tetraphenylporphyrin substituted at position 4 of one of the phenyl rings as *e.g.*, with an amino or aldehyde group [6, 7]. Two strategies were followed: first, the direct attachment of the *meso*-tetraphenylporphyrin moiety to the methyl carbon atom of the hypericin core, and second, hybridization of the two chromophores *via* a linker durable against hydrolytic cleavage.



1 **2**

Formulae 1

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Results and discussion

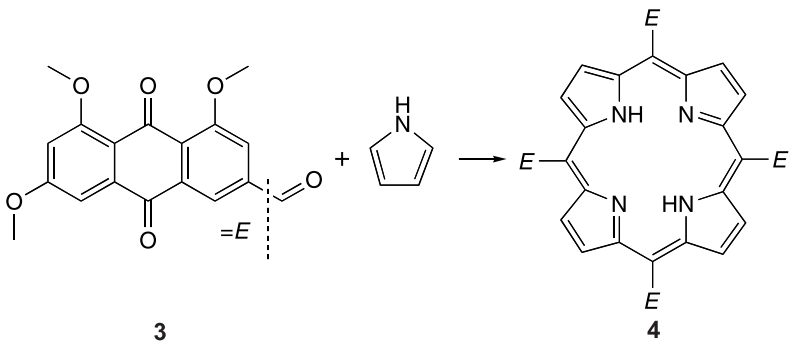
Syntheses

Following the first approach, the methyl-protected emodinaldehyde **3** (prepared by a *Sommelet* reaction from the easily available tri-*O*-methyl-emodin-ω-bromide [8]) was condensed with an equimolar amount of pyrrole in refluxing propionic acid/propionic anhydride according to the conventional synthesis of tetraarylporphyrines [9] to provide the tetra-*meso*-anthraquinoylporphyrin derivative **4** in rather low yield. Interestingly enough, according to its ^{1}H NMR spectrum, this derivative consists of a mixture of the diastereomers generated from the restricted rotation around the *meso*-methine linkages. Unfortunately however, **4** neither could be reduced to the corresponding anthrone derivative, nor could it be reacted with emodinanthrone in the recently established microwave-induced condensation [10] to yield the corresponding hypericin-porphyrin hybrid. The latter failure is probably due to steric reasons.

Thus, we followed the second approach. In exploratory syntheses it turned out that derivatives with a linkage between emodin (and in the subsequent step, emodinanthrone) and a tetraphenylporphyrin by means of a propionic or acrylic unit with an amidic bond could not be dimerized in the usual manner, obviously also due to steric reasons. Thus, we targeted a mono-substituted system, and in addition, turned to a purely aliphatic linkage (providing outmost stability against hydrolytic cleavage) employed earlier to attach phenyl derivatives to the ω-positions of hypericin [11, 12]. Upon *Wittig* reaction between the *O*-methyl-protected emodin-phosphonium salt **5** [8] and the *meso*-tetraphenylporphyrinaldehyde **6** [4] in presence of K_2CO_3 and 18-crown-6 with dichloromethane as the solvent, a 1:1 mixture (72%) of the corresponding diastereomers (*Z*)- and (*E*)-**7** was obtained. The two diastereomers could be chromatographically separated, characterized, and assigned with respect to their diastereomerism based on the typical coupling constants of 12 and 16 Hz for the protons of the ethylene fragment. Conventional reduction by $SnCl_2$ [13] provided the anthrones (*Z*)- and (*E*)-**8** in yields of about 70%. The latter compounds were treated in a microwave-induced reaction with emodin in presence of potassium *tert*-butanolate according to Ref. [10] to give the diastereomeric protohypericin derivatives (*Z*)- and (*E*)-**9** in yields of about 80%, which were subsequently photocyclized to the hypericin-porphyrin hybrid target compounds (*Z*)- and (*E*)-**10** in nearly 90% yields.

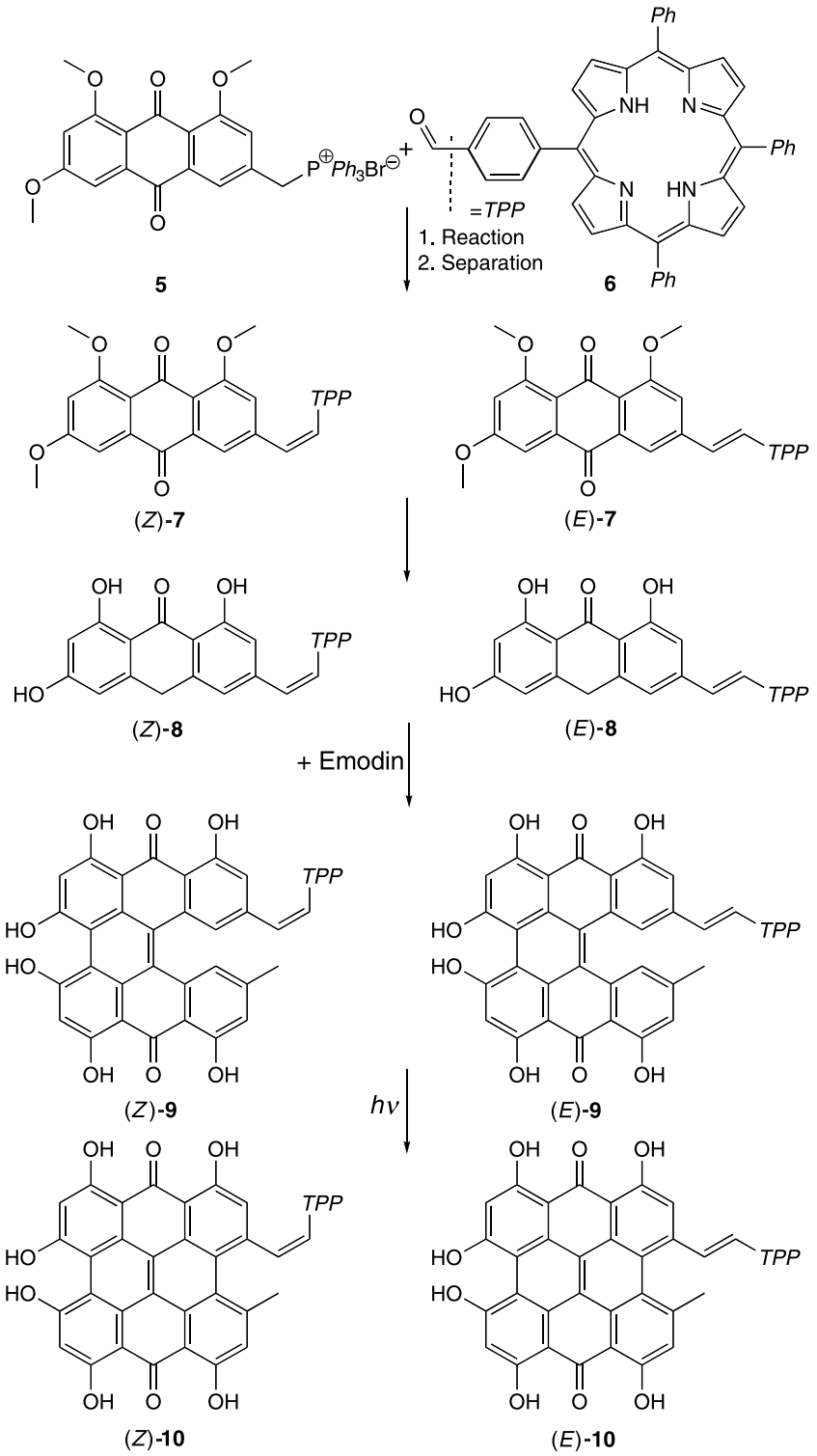
*Properties of the hypericin-porphyrin-hybrids (Z)- and (E)-**10***

The two most interesting points from the view of photodynamic therapy is the absorption spectrum of the compounds and their ability to sensitize the formation of singlet oxygen and/or reactive oxygen species. Whereas the porphyrinic *Soret* bands of both diastereomers remained un-shifted at 414 nm and the Q-bands are only slightly affected, the hypericin band is bathochromically shifted from 586 nm for (*Z*)-**10** to 618 nm for (*E*)-**10**, which for the latter is still a bathochromic shift of about 20 nm from the absorption wavelength of hypericin (**1**) itself. Accordingly, (*Z*)-**10** is a rather sterically crowded ethylene derivative (see Fig. 1 for the structures of the two diastereomers as obtained from semiempirical calculations) with severely restricted



3 **4**

Scheme 1



Scheme 2

conjugation between the two chromophoric units, where even a hypsochromic shift of the hypericin band compared to **1** is very remarkable. The fluorescence properties of the two diastereomers are rather complicated by the fact that the emissions are dependent on the excitation wavelengths pointing to dif-

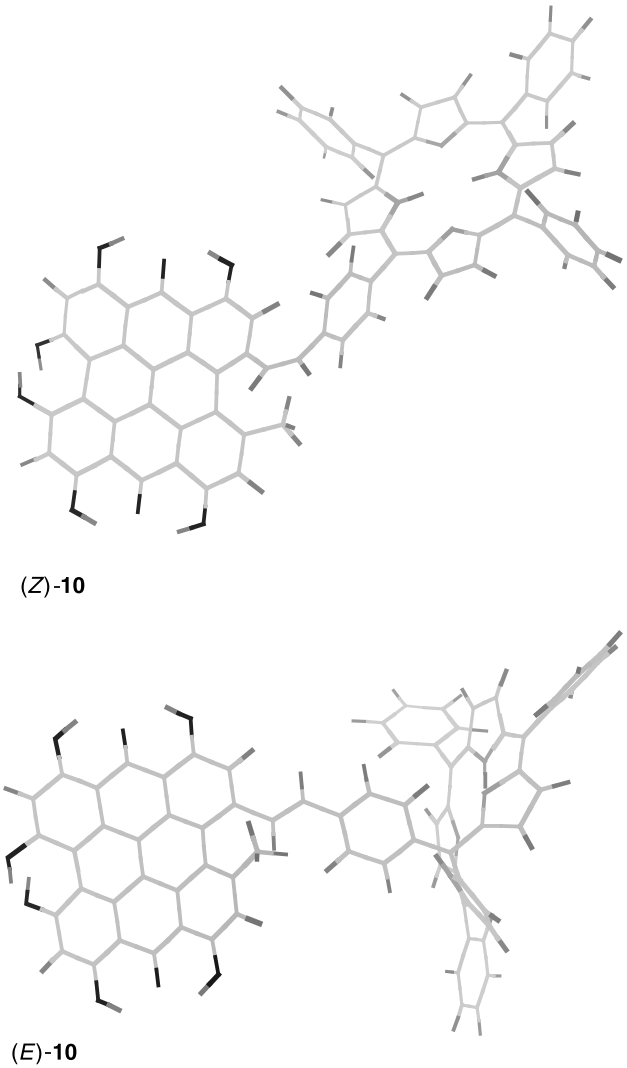


Fig. 1 Structures of (*Z*)- and (*E*)-**10** as derived from MM2 calculations

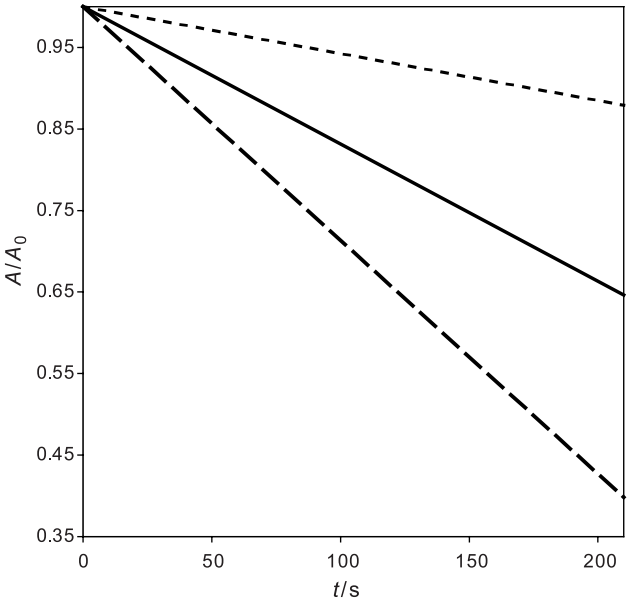


Fig. 2 Hypericin derivative sensitized photooxidation of bilirubin IXα: normalized absorption (A/A_0) *vs.* time curves of solutions of disodium bilirubinate IXα together with the sodium salts of either hypericin (**1**, –) and hypericin-porphyrin hybrid diastereomers (*Z*)-**10** (– – –) and (*E*)-**10** (. . .)

ferent ways of excitation transfers between the two sub-chromophors.

With respect to the ability of (*Z*)- and (*E*)-**10** to photosensitize the formation of singlet oxygen and/or reactive oxygen species we investigated their photo-destructing power against bilirubin IXα as established earlier [14]. Figure 2 demonstrates that the (*Z*)-diastereomer of **10** is much more potent than hypericin (**1**), whereas the (*E*)-diastereomer is less active. Obviously, the strong decoupling of the two chromophores in the (*Z*)-diastereomer gives the photosensitizing activity a strong impetus, whereas in the (*E*)-diastereomer the more or less extensive conjugation between the two chromophors is contra-productive for this effect. Thus, the bathochromic shift of (*E*)-**10** is somewhat counterfeited by the lower sensitizing properties. On the other hand, the high photosensitizing activity of the (*Z*)-diastereomer is a little hampered by its slight hypsochromic shift compared to **1**. In addition to this shortcoming, a certain amount of photodestruction of (*Z*)-**10** as indicated by the bleaching of the *Soret* band absorption was observed – however, this is an effect that could also become a bonus with respect to the photo-therapeutic use: the compound is removed during irradiation from the site of destruction and could do no further harm in surrounding healthy tissue. Obviously, decoupling of the conjugation between the two chromophoric units assuring an un-shifted long-wavelength band of the hypericin moiety and optimal sensitizing properties would be a promising target.

Conclusions

Two diastereomeric hypericin-tetraphenylporphyrin hybrids could be prepared, which are characterized by an ethylenic conjugated linker between them. They displayed significantly distinct photosensitization properties with the (*Z*)-configured derivative

more active than hypericin itself. Accordingly, the hybridization of hypericin and porphyrin chromophores, in particular, when they are electronically decoupled, seems to be a promising road in the development of second/third-generation photosensitizers for photodynamic therapy.

Experimental

The characterizations of the products were established using mp (Kofler microscope, Reichert), ^{1}H NMR (Bruker DPX 200 and 500 MHz), IR (Bruker Tensor 27, KBr), MS (Thermofinnigan LCQ Deca XP Plus), fluorescence (Varian *Carey* Eclipse fluorescence instrument), and UV-Vis data (Varian Cary 100 Bio). Fluorescence quantum yields were calculated according to the comparative method of *Williams et al.* [15] using hypericin (**1**) as standard sample. Microwave assisted syntheses were performed on a MLS-ETHOS 1600 microwave unit with Terminal 320 from MLS-Milestone with temperature monitoring and control. The production of singlet oxygen/oxidizing species by (*Z*)-**10** and (*E*)-**10** was monitored by bilirubin-IXα-degradation according to Ref. [14]. The long-wavelength "hypericinic" absorption band intensities of the two compounds were made equal by adjusting concentrations to provide comparable light absorptions for the two compounds. Emodin was obtained from *Cortex frangulae* as described in Ref. [16]. 1,3,8-Trimethoxy-9,10-anthraquinon-6-carbaldehyde (**3**) was prepared according to Ref. [8].

meso-Tetrakis(1,3,8-trimethoxy-9,10-anthraquinon-6-yl)-porphyrin (**4**, $C_{89}H_{74}N_4O_{20}$)
To a solution of 500 mg aldehyde **3** (1.53 mmol) in 25 cm^3 propionic acid and 250 mm^3 propionic anhydride 107 mm^3 pyrrole (1.56 mmol) were added at 140°C. After refluxing for 3 h the solution was cooled to room temperature. The propionic acid was evaporated *in vacuo* and the product mixture was extracted with $CHCl_3$/aqu $NaHCO_3$. The resulting residue was purified on silica gel, whereby the purple fractions were collected yielding 73 mg **4** (3.5% based on **3**). Mp > 320°C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = -1.95$ (s, 2NH), 3.85–4.00 (m – due to restricted rotation at the methane bonds, several diastereomers are present, 12O*Me*), 6.70–8.90 (m, 20H-ar) ppm; IR (KBr): $\bar{\nu} = 3420$, 2932, 2836, 1717, 1699, 1595, 1457, 1319, 1250, 1135, 1250, 1135, 1069, 996, 946, 929, 880, 800, 755, 668, 566 cm^{-1}; UV-Vis (acetone): $\lambda_{max} = 277$ (50), 432 (100), 520 (7), 558 (2), 593 (1), 648 (1) nm (rel. int.).

(Z)-1-(1,3,8-Trimethoxy-9-10-anthraquinon-6-yl)-2-(meso-tetraphenylporphyrin-4-yl)ethene and ((Z)-7, $C_{63}H_{44}N_4O_5$) *and (E)-1-(1,3,8-Trimethoxy-9-10-anthraquinon-6-yl)-2-(meso-tetraphenylporphyrin-4-yl)ethene* ((*E*)-**7**, $C_{63}H_{44}N_4O_5$)
Phosphoniumbromide **5** (0.3 g, 3.06 mmol, prepared according to Ref. [8a]), 0.14 g dried K_2CO_3 (6.1 mmol), and 0.1 g 18-crown-6 (0.405 mmol) were taken up in 25 cm^3 absolute CH_2Cl_2 and refluxed for 10 min. Then, a solution of 0.288 g 5-(4-formylphenyl)-10,15,20-triphenylporphyrin (**6**) (0.45 mmol) prepared according to Ref. [4] in 40 cm^3 CH_2Cl_2 were added drop-wise over a period of 30 min. The reaction solution was refluxed for 5 h, cooled, and extracted three times with $CHCl_3/H_2O$. The organic layer was evaporated to dryness giving 0.39 g (2.18 mmol) of a mixture of the diastereomers. These were separated on silica gel with $CHCl_3$:CH_3OH = 15:1 as the mobile phase.

(*Z*)-**7**: Yield 36%; mp 205–209°C; TLC: $R_f = 0.25$ ($CHCl_3$); ESI-MS (CH_3OH:$CHCl_3$ = 10:1, positive ion mode): m/z = 937 ($[M+H]^+$); IR (KBr): $\bar{\nu} = 3314$, 2929, 1771, 1667, 1593, 1456, 1440, 1348, 1317, 1249, 1220, 1202, 1153, 1128, 1070, 1019, 1001, 979, 965, 876, 798, 751, 726, 700, 657 cm^{-1}; UV-Vis ($CHCl_3$): $\lambda_{max} = 415$ (100), 512 (3), 548 (2), 591 (1) nm (rel. int.); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = -1.8$ (s, 2H), 3.85, 3.92, 3.98 (3s, 3O*Me*), 6.85 (d, J = 12 Hz, =CH), 7.17 (d, J = 12 Hz, =CH), 7.27–8.90 (m, 23H-ar) ppm.

(*E*)-**7**: Yield 36%; mp 205–209°C; TLC: $R_f = 0.21$ ($CHCl_3$); ESI-MS (CH_3OH:$CHCl_3$ = 10:1, positive ion mode): m/z = 937 ($[M+H]^+$); IR (KBr): $\bar{\nu} = 3313$, 2925, 2883, 1771, 1700, 1593, 1456, 1440, 1348, 1317, 1220, 1202, 1153, 1128, 1070, 1019, 1001, 979, 965, 798, 751, 726, 700, 657 cm^{-1}; UV-Vis ($CHCl_3$): $\lambda_{max} = 415$ (100), 512 (3), 548 (2), 591 (1) nm (rel. int.); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = -1.8$ (s, 2H), 3.85, 3.92, 3.98 (3s, 3O*Me*), 7.68 (d, J = 16 Hz, =CH), 7.17 (d, J = 16 Hz, =CH), 7.27–8.90 (m, 23H-ar) ppm.

(Z)-1-(1,3,8-Trihydroxy-9-anthracene-10-on-6-yl)-2-(meso-tetraphenylporphyrin-4-yl)ethene ((*Z*)-**8**, $C_{63}H_{44}N_4O_5$)
To a solution of 132 mg (*Z*)-**7** (0.141 mmol) in 11 cm^3 glacial acetic acid 5 cm^3 HBr (47%) and 254 mg $SnCl_2 \cdot 2H_2O$ (1.22 mmol) were added at 70°C under an Ar atmosphere. After 1 h at this temperature the mixture was poured on ice and neutralized with 1 *N* NaOH. The solid was extracted with ethyl acetate and dried during the night over P_2O_5 to yield 90 mg (73%) (*Z*)-**8** as a violet solid. Mp > 320°C; TLC: R_f = 0.69 ($CHCl_3$); NCI-MS (solid probe, CH_4): m/z = 881 ($[M]^+$); IR (KBr): $\bar{\nu} = 3314$, 2929, 1771, 1667, 1593, 1456, 1440, 1348, 1317, 1249, 1220, 1202, 1153, 1128, 1070, 1019, 1001, 979, 965, 876, 798, 751, 726, 700, 657 cm^{-1}; UV-Vis (acetone): $\lambda_{max} = 416$ (100), 512 (1), 548 (1), 592 (1), 648 (1) nm (rel. int.); due to its air sensitivity no ^{1}H NMR could be recorded.

(E)-1-(1,3,8-Trihydroxy-9-anthracene-10-on-6-yl)-2-(meso-tetraphenylporphyrin-4-yl)ethene ((*E*)-**8**, $C_{60}H_{40}N_4O_4$)
To a solution of 35 mg (*E*)-**7** (0.0374 mmol) in 3 cm^3 glacial acetic acid heated to 80°C under Ar, 1.3 cm^3 HBr (47%) and then 67 mg $SnCl_2 \cdot 2H_2O$ (0.323 mmol) were added. The resulting mixture was refluxed for 60 min, poured on ice, and neutralized with aqu $NaHCO_3$. After the precipitate was extracted with ethyl acetate the solvent was removed and the residue dried over P_2O_5 during the night to yield 30 mg (93%) (*Z*)-**8** as a purple solid. Mp > 320°C; TLC: $R_f = 0.67$ ($CHCl_3$); IR (KBr): $\bar{\nu} = 3314$, 2929, 1771, 1667, 1593, 1456, 1440, 1348, 1317, 1249, 1220, 1202, 1153, 1128, 1070, 1019, 1001, 979, 965, 876, 798, 751, 726, 700, 657 cm^{-1}; UV-Vis (acetone): $\lambda_{max} = 348$ (13), 415 (100), 512 (1), 547 (1), 590

(1), 648 (1) nm (rel. int.); due to its air sensitivity no ^{1}H NMR could be recorded.

(Z)-1-meso-Tetraphenylporphyrin-4-yl-2-(1,8,10,12,13,15-hexahydroxy-dibenzo[oa]perylene-3-yl-)ethene
((*Z*)-**9**, $C_{75}H_{46}N_4O_8$)
Emodin (18 mg, 0.066 mmol), 59 mg (*Z*)-**8** (0.066 mmol), 10 mg K *tert*-butoxide (0.0114 mmol), and 0.5 cm^3 *DMF* were mixed in a round-bottom flask and irradiated in the microwave unit at 150 W for 30 min. The reaction mixture was quenched with water and acidified with 2 *N* HCl. The precipitate was centrifuged and dried over P_2O_5 over night under vacuum. Yield 70 mg (85%); mp > 320°C; NCI-MS (solid probe, CH_4): $m/z = 1129$ ($[M]^+$); UV-Vis (acetone): $\lambda_{max} = 414$ (100), 512 (7), 544 (7), 586 (12), 648 (2) nm (rel. int.); due to its light and air sensitivity no ^{1}H NMR could be recorded.

(E)-1-meso-Tetraphenylporphyrin-4-yl-2-(1,8,10,12,13,15-hexahydroxy-dibenzo[oa]perylene-3-yl-)ethene
((*E*)-**9**, $C_{75}H_{46}N_4O_8$)
A mixture of 20 mg emodin (0.074 mmol), 65 mg (0.073 mmol) (*E*)-**8**, 10 mg K *tert*-butoxide (0.089 mmol), and 0.5 cm^3 *DMF* was irradiated in the microwave unit for 20 min at 150 W under Ar. The mixture was molten within the first minute followed by a gentle reflux for the rest of the time. After cooling the mixture was taken up with water and acidified with 2 *N* HCl and extracted with $CHCl_3$. The organic layer was concentrated and the residue purified on silica gel ($CHCl_3$:CH_3OH = 4:1). The hypericin fractions were collected giving 58 mg (70%) of the product mp > 320°C; NCI-MS (solid probe, CH_4): $m/z = 1129$ ($[M]^+$); UV-Vis (acetone): $\lambda_{max} = 414$ (100), 513 (5), 571 (6), 618 (12), 648 (1) nm (rel. int.); due to its light and air sensitivity no ^{1}H NMR could be recorded.

(Z)-1-meso-Tetraphenylporphyrin-4-yl-2-(1,6,8,10,11,13-hexahydroxy-7,14-dioxo-7,14-dihydrophenanthro[1,10,9,8-opqra]perylene-3-yl-)ethene ((*Z*)-**10**, $C_{75}H_{44}N_4O_8$)
A solution of 20 mg (*Z*)-**9** in 300 cm^3 acetone was irradiated for 10 min by means of a 700 W Hg high-pressure lamp with fluorescence screen (*Philips*) under stirring and air admission. After evaporation of the solvent the resulting purple solid was triturated with acetone to yield 17 mg (85%) of the product. Mp > 320°C; NCI-MS (solid probe, CH_4): $m/z = 1129$ ($[M]^+$); IR (KBr): $\bar{\nu} = 3420$, 3055, 2923, 1577, 1472, 1419, 1277, 1183, 965, 800, 701 cm^{-1}; UV-Vis (acetone): $\lambda_{max} =$ 414 (100), 512 (7), 544 (7), 586 (12), 648 (2) nm (rel. int.); fluorescence (ethanol, $\lambda_{ex} = 414$ nm): $\lambda_f = 650$ (1.0), 710 (0.5) nm (rel. int.); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = -1.8$ (s, 2NH), 2.10 (s, CH_3), 5.0 (d, $J = 12$ Hz, =CH), 5.7 (d, $J =$ 12 Hz, =CH), 8–10 (m, 23H-ar), 15–18 (br, 6OH) ppm.

(E)-1-meso-Tetraphenylporphyrinyl-2-(1,6,8,10,11,13-hexahydroxy-7,14-dioxo-7,14-dihydrophenanthro[1,10,9,8-opqra]perylene-3-yl-)ethene ((*E*)-**10**, $C_{75}H_{44}N_4O_8$)
A solution of 24 mg (*E*)-**9** in 300 cm^3 acetone was irradiated for 10 min by means of a 700 W Hg high-pressure lamp with fluorescence screen (Philips) under stirring and air admission. After evaporation of the solvent the resulting purple solid was triturated with acetone to yield 22 mg (91%) of the product. Mp > 320°C; NCI-MS (solid probe, CH_4): $m/z = 1129$ ($[M]^+$); IR (KBr): $\bar{\nu} = 3420$, 3055, 2923, 1577, 1472, 1419, 1277, 1183, 965, 800, 701 cm^{-1}; UV-Vis (acetone): $\lambda_{max} = 414$ (100), 513 (5), 571 (6), 618 (12), 648 (1) nm (rel. int.); fluorescence (ethanol, $\lambda_{ex} = 414$ nm): $\lambda_f = 630$ (0.9), 650 (1.0), 710 (0.5) nm (rel. int.); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = -1.8$ (s, 2NH), 2.10 (s, CH_3), 5.2 (d, $J = 16$ Hz, =CH), 5.9 (d, $J = 16$ Hz, =CH), 8–10 (m, 23H-ar), 14–16 (br, 6OH) ppm.

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