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Synthesis and Properties of Hypericins Substituted with Acidic and Basic Residues: Hypericin Tetrasulfonic Acid – A Water Soluble Hypericin Derivative

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Summary. Sulfonation of hypericin leads to its di-, tri-, and tetrasulfonic acid derivatives. The latter is soluble in water up to millimolar solutions. Homoaggregate formation (*J*-aggregates) was observed only above $5 \cdot 10^{-4}$ mol/l. In water solutions, the tetrasulfonic acid hypericin derivative was found to be present as its *bay*-phenolate with most of the sulphonic acids dissociated. Thus, the first water soluble hypericin derivative, which in contrast to hypericin is not prone to homoassociation, is presented. Hypericin tetrtasulfonic acid forms heteroassociates with serum albumin, DNA, and γ-cyclodextrin. Hypericin derivatives with primary and tertiary amino group appendages at the hypericin methyl groups were synthesized. However, upon salt formation or quaternization these derivatives became virtually insoluble in all common solvents including water.

Keywords. Hypericin sulfonic acids; Appended primary and tertiary amino hypericins; *J*-Aggregates; Heteroassociates.

Synthese und Eigenschaften von mit sauren und basischen Resten substituierten Hypericinen: Hypericintetrasulfonsäure – ein wasserlösliches Hypericinderivat

Zusammenfassung. Sulfonierung von Hypericin führt zu seinen Di-, Tri- und Tetrasulfonsäurederivaten. Letzteres ist in Wasser bis zu millimolaren Konzentrationen löslich. Homoaggregatbildung (*J*-Aggregate) wurde erst ab Konzentrationen von $5 \cdot 10^{-4}$ Mol/l beobachtet. Die Tetrasulfonsäure liegt in wäßriger Lösung als *bay*-Phenolat vor, wobei die meisten Sulfonsäuregruppen dissoziiert sind. Damit wurde das erste wasserlösliche Hypericinderivat, das im Gegensatz zum Hypericin nicht zur Homoassoziation neigt, gefunden. Hypericintetrasulfonsäure bildet Heteroassociate mit Serumalbumin, DNA und γ-Cyclodextrin. Es wurden weiters Hypericinderivate synthetisiert, die über primäre und tertiäre Aminogruppen im Bereich der Methylgruppen des Hypericins verfügen. Bei Salzbildung oder Quarternierung werden diese Derivate jedoch in allen üblichen Lösungsmitteln, inklusive Wasser, praktisch unlöslich.

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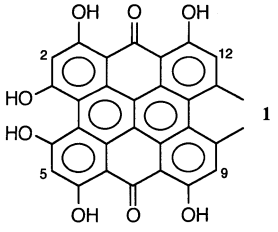
Introduction

Hypericin (**1**) and most of its derivatives and structural relatives are known for their intrinsically low solubility in most common solvents, especially in water. Since the

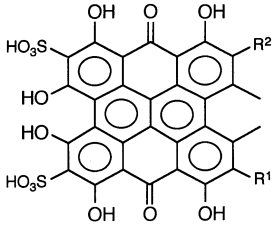


latter solvent is fundamental with respect to physiological and therapeutic conditions, the search for hypericin derivatives of enhanced water solubility is an important objective. Recently we have been able to enhance the solubility of hypericin in water by attaching long chains of polyethylene glycols to its two methyl groups [1]. However, although the solubility of the *bis*-ω,ω'-polyethylene-glycol-1000 hypericin derivative in water has been enhanced to about $5 \cdot 10^{-5}$ mol/l, it started to homoassociate into *H*-aggregates at concentrations as low as 10^{-6} mol/l [1]. Therefore, the quest for water soluble derivatives, preferable compounds without association tendency, was pursued again using derivatives with acidic and basic functional groups. Following the lead of color chemistry [2] to enhance water solubility, the preparation of hypericin sulfonic acids (which allow the formation of sulfonic acid salts) and amines (which allow the formation of ammonium salts and quaternary ammonium salts) was set as a goal and will be described in this paper.

Results and Discussion

Hypericin sulfonic acids

Since it has been established recently that hypericin (**1**) could be substituted stepwise by means of electrophilic bromination to yield regioselectively 2,5-dibromohypericin, 2,5,9-tribromohypericin, and 2,5,9,12-tetrabromohypericin [3], electrophilic sulfonation seemed to be promising in this context.



2 : $R^1 = R^2 = \mathrm{H}$
3 : $R^1 = \mathrm{SO_3H},\ R^2 = \mathrm{H}$
4 : $R^1 = R^2 = \mathrm{SO_3H}$

Unfortunately, the photochemical sulfonation of **1** using Na_2SO_3 in aqueous pyridine gave only unreacted **1**. However, upon sulfonation of **1** with oleum at room temperature it turned out (monitoring by NMR) that the sulfonation proceeded regioselectively and stepwise into the aromatic positions 2/5 and 2/5/9, thus providing the corresponding disulfonic and trisulfonic hypericin derivatives **2** and **3** in 27% yield. The rest to the reaction mixture proved to be unreacted **1**. Therefore, the reaction temperature was raised to 78°C in the next experiment and

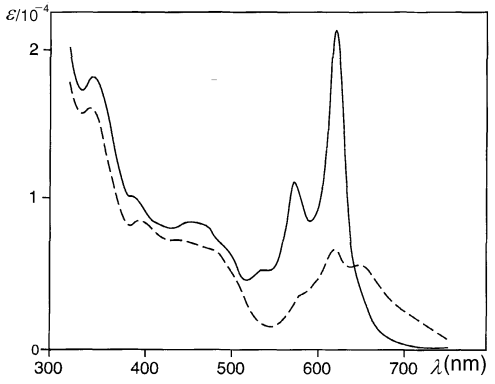


Fig. 1. Concentration dependence of the absorption spectrum of **4** in water; c from $5 \cdot 10^{-7}$ to $2 \cdot 10^{-4}$ mol/l (–), $c = 1 \cdot 10^{-3}$ mol/l (- - -)

kept for one hour. This resulted in a mixture of **2**, **3** and tetrasulfonic acid **4**, with **2** and **4** as the minor components. The major product **3** could be isolated by chromatography on Sephadex in 58% yield and was characterized. The tetrasulfonic acid **4** was then prepared in 86% yield by prolonging the reaction time to about 1.5 hours under the conditions given above. Although the water solubility of **2** and **3** was somewhat enhanced in relation to **1**, the tetrasulfonic acid **4** displayed by far the best water solubility. Therefore we focused upon preparation, characterization, and properties of **4**.

The tetrasulfonic acid **4** proved to be soluble in water up to concentrations of about 10^{-3} mol/l. As demonstrated in Fig. 1, the absorption spectrum of **4** in water at concentrations between $5 \cdot 10^{-7}$ to $2 \cdot 10^{-4}$ displays the characteristic features of a monomolecularly dissolved hypericinate species mol/l [4]. Compared to **1**, the long wavelength band was bathochromically shifted to 615 nm. For concentrations above $5 \cdot 10^{-4}$ mol/l and up to the solubility limit of about 10^{-3} mol/l, a bathochromically shifted shoulder developed at 660 nm and became gradually more intense on the expense of the band at 615 nm. In contrast to the hypericin-water system which is characterized by a *H*-aggregate [4], this species could constitute a *J*-aggregate. However, it could not be ruled out that it is simply an undissociated species since addition of *Hünig*'s base to a concentrated solution recovered the low concentration species. Nevertheless, the rather broad long wavelength absorption band together with its low intensity and a hypericinate type short wavelength band system favor the first explanation. The fluorescence spectrum of **4** in water peaked at 625 nm, exhibiting a slightly larger *Stokes* shift (10 nm) than that observed for the parent system **1** dissolved in ethanol (which, exactly speaking, is that of the *bay*-hypericinate ion in both cases). The fluorescence quantum yield of 0.8 was found to be considerably enhanced compared to that of **1** dissolved in *DMSO* [4]. It should be noted that dissolving **1** in water by means of sonication or solubilization with *DMSO* renders the compound to be non-fluorescent [4].

Of course, the protonation-deprotonation equilibria of **4** are rather complex due to the presence of the rather acidic *bay*-hydroxylic groups (the corresponding pK_a of **1** has been deduced to amount to 1.8 [1]) and the four sulfonic acid groups (the pK_a of benzene sulfonic acid has been determined to amount to 0.7 [5]). Nevertheless, with respect to the hypericin chromophore the C=O-protonated, the

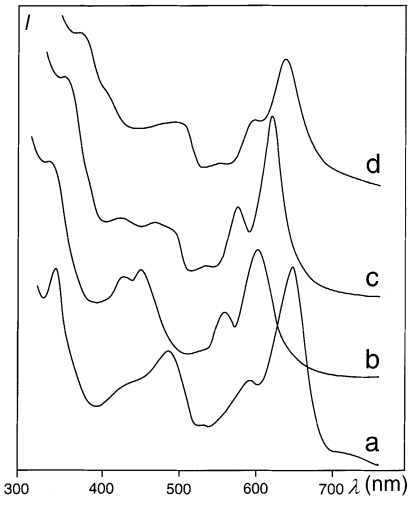


Fig. 2. Absorption spectra of **4** dissolved in water ($c = 2 \cdot 10^{-5}$; arbitrary E values displaying correct relative band intensities) at $H_- = -6.5$ (a), $pH = 0$ (b), $pH = 5$ (c), and $pH = 10$ (d); fluorescence peaks in these cases were observed at 668 (a), 610 (b), 625 (c), and 625 (d) nm

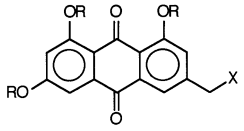
undissociated, the *bay*-hypericinate, and the *bay-peri*-hypericindiate species could be detected upon spectrophotometric titration (Fig. 2: traces a–d). For the corresponding steps, pK_a values of -6, 3.8, and 8.8 were estimated. A potentiometric titration yielded only one very broad cumulative step up to *pH* 7. Although the dissociation of the sulfonic acid groups play a negligible role for the spectroscopic shifts of the various species, these results point to a decrease of the *bay*-hydroxyl acidity induced by the adjacent dissociated sulfonic acid group(s).

The absorption spectrum of **4** in dimethylsulfoxide was found to be superimposable on that of its water solution at low concentrations (Fig. 1). However, in other common organic solvents, like methanol, ethanol, acetonitrile, acetone, and tetrahydrofuran, the solubility of **4** was exceptionally low, and the absorption spectra of such only faintly colored solutions displayed severely broadened and bathochromically shifted long wavelength bands resembling the high concentration species of Fig. 1. An exception was the pyridine solutions of **4** which displayed the characteristic hypericinate type spectrum.

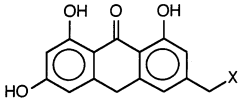
The tetrasulfonic acid **4** formed heteroassociates with bovine and human serum albumin (BSA and HSA), DNA, and γ-cyclodextrine as indicated by a sigmoidal shift *vs.* concentration dependence of the long wavelength absorption band upon gradual addition of these compounds. Thus, this band was shifted from 615 nm to 621 and 619 nm with BSA and HSA displaying saturation behaviour above the concentration threshold of **4**: (B)HSA = 1:2. However, in contrast to the HSA complex of **1** [4], no chiroptical signal of the hypericin chromophore could be detected. Bilirubin and warfarin were not displaced by **4** from their HSA complexes. Although the HSA complex of **4** could be chromatographed it seemed to be less stable than the corresponding hypericin complex, and the complexation seemed to be non-specific. In the case of DNA, a continuous shift to about 625 nm was observed; however, no chiroptical signal for the hypericin chromophore could be detected. Complexation with γ-cyclodextrin seemed to be rather limited since a shift of the long wavelength band of **4** to 618 nm was observed only upon addition of a 270 fold excess. Again, no chiroptical signal could be detected.

Amino derivatives of hypericin

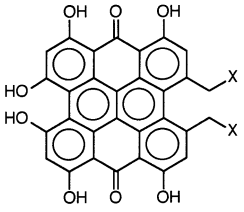
To attach an amino group to the hypericin moiety, it was decided to prepare a primary and a tertiary amine functionality somewhat spaced from the strongly acidic *bay* region. Thus, we resorted to the strategy applied to the synthesis of a variety of ω,ω'-appended hypericin derivatives [6].



5 : $R = CH_3CO$, $X = OCH_2CH_2NHCOCH_3$
6 : $R = H$, $X = OCH_2CH_2NHCOCH_3$
10 : $R = H$, $X = OCH_2OCH_2Cl$



7 : $X = OCH_2CH_2NHCOCH_3$
11 : $X = OCH_2OCH_2Cl$



8 : $X = OCH_2CH_2NHCOCH_3$
9 : $X = OCH_2OCH_2NH_2$
12 : $X = OCH_2CH_2NH(CH_2)_5$

Accordingly, **5** was prepared from the recently described ω-iodo-tri-O-acetoxy emodin [7] by means of a nucleophilic displacement with N-(2-hydroxyethyl) acetamide in presence of Ag*Tf* in 37% yield. Acidic saponification (90%) to yield **6** and reduction (90%) afforded the corresponding anthron **7** which was then coupled and photocyclized to the appended hypericin derivative **8** in only 10% yield. Upon acidic saponification, the primary amino derivative **9** was produced. However, it proved to be virtually insoluble in common solvents. Experiments to quarternize **9** with CH_3J also failed to yield any soluble material.

To prepare a tertiary amino derivative of **1**, ω-bromo-tri-O-acetoxy emodin [6] was substituted by means of 2-chloroethanol in presence of silver perchlorate to yield **10** (95%). Upon reduction, the anthron **11** was obtained (90%) which was then dimerized in presence of an excess of piperidine to yield 7% of the *bis*-piperidinyl derivative **12**. This compound displayed the usual characteristic spectroscopic features of a hypericinate derivative. It was obviously present as its zwitterion with respect to the *bay* hydroxyl group. Judged from its absorption spectrum, the second amino group was insufficient, however, to ionize an additional *peri* positioned hydroxyl group. It displayed marginal water solubility, and upon protonation or quaternization of the nitrogen atoms it became virtually insoluble in all common solvents. This phenomenon might be due to the intrinsic insolubility of nonionized hypericin.

Conclusions

Two routes to enhance the water solubility of hypericin without changing its functional groups were pursued. On the one hand, tetrasulfonation of hypericin provided the ideal means to enhance the water solubility of hypericin even into the

millimolar region. This should be sufficient for any biological purposes. The interactions of this material with certain biopolymers was shown to result in heteroassociation complexes. On the other hand, although amino derivatives of hypericin are obtainable in principle, they yield virtually insoluble salts upon protonation or quaternization and are thus unsuited for further physiological studies.

Experimental

Melting points were taken by means of a Kofler hot stage microscope (Reichert, Vienna). ^{1}H, ^{13}C, IR, UV/Vis, fluorescence, CD, and mass spectra were recorded using Bruker DPX 200 and 500, Biorad FT-IR-45, Perkin-Elmer IR-710B, Hitachi-U-3210 and F-4010, ISA-mark-V, and API-5989 instruments. Spectrophotometric titrations (dist. water) were carried out as described in Ref. [1]. For the determination of the fluorescence quantum yields, Rhodamine B fluorescence ($\Phi_f = 0.69$; ethanol) was used as the standard. Hypericin (**1**) was prepared according to Ref. [8] Bovine and human serum albumin, calf thymus DNA, and γ-cyclodextrin were of commercial origin (Sigma). Heteroassociation was studied spectrophotometrically in phosphate buffer solutions of *pH* 7 as described in Ref. [4].

Sulfonation procedure

41 mg **1** and 125 mg oleum were kept at the temperature and time given. The reaction mixture was gently diluted with ice water and saturated with NaCl. After centrifugation, the precipitated green crystals were washed with cold water and thoroughly dried in vacuum. The material in question was isolated by column chromatography on Sephadex-LH20 with methanol/water (4/1) as the eluent.

Hypericin-2,5-disulfonic acid (**2**; $C_{30}H_{16}O_{14}S_2$)

Proceeding with the solfonation procedure given above with 32 mg **1** and 80 mg oleum at room temperature for 1.5 h, 12 mg **2**+**3** which could not be separated properly were obtained. From this mixture, the ^{1}H NMR data of **2** could be derived ^{1}H NMR (*DMSO*-d_6, δ, 500 MHz): 15.00 (s, 2H), 14.36 (s, 2H), 13.51 (s, 2H) 7.48 (s, CH-9, 12), 2.74 (s, 6H, CH_3-10, 11) ppm.

Hypericin-2,5,9-trisulfonic acid (**3**; $C_{30}H_{16}O_{17}S_3$)

Proceeding with the sulfonation procedure given above with 41 mg **1** and 125 mg oleum at 78°C for 1 h, 35 mg (58%) **3** were obtained.

M.p.: not below 300°C; ^{1}H NMR (*DMSO*-d_6, δ, 500 MHz): 17.38 (s, 1H), 15.04 (s, 1H), 14.34 (s, 1H), 14.12 (s, 1H), 13.96 (s, 1H), 13.47 (s, 1H), 7.44 (s, CH-9), 2.98 (s, CH_3-11), 2.71 (s, CH_3-10) ppm; ^{13}C NMR (50.3 MHz): due to its low solubility, the spectrum was not properly resolved; however, two C=O signals at 197 and 195 ppm were significant; IR (KBr): $\nu = 3426$, 1595, 1447, 1359, 1235, 1036, 823 cm^{-1}; MS (*e*-spray ionization from water/acetonitrile = 1/1): $m/z = 744$ (10; M^+), 663 (30), 474 (8), 457 (7), 371 (76), 331 (1000), 247 (91), 220 (30), 169 (8), 117 (24); UV/Vis (H_2O, $c = 3 \cdot 10^{-6}$ mol/l): $\lambda_{max} = 605$ (22000), 570 (15000), 449 (15000), 318 (30000) nm (ε); fluorescence (H_2O, $c = 3 \cdot 10^{-6}$): $\lambda_{em} = 617$ (1), 660 (0.4, sh) nm (rel. intensity).

Hypericin-2,5,9,12-tetrasulfonic acid (**4**; $C_{30}H_{16}O_{20}S_4$)

Proceeding with the sulfonation procedure given above with 50 mg **1** and 250 mg oleum at 75°C for 1.3 h, 78 mg (95%) **4** were obtained. Elemental analysis showed, however, that due to the salting

out procedure the material could be contaminated even after chromatography by as much as 75% with NaCl.

M.p.: not below 300°C; ^{1}H NMR (*DMSO*-d_6, δ, 500 MHz): 17.34 (s, 2H), 14.05 (s, 2H), 14.03 (s, 2H), 2.96 (s, CH_3-10, 11) ppm; ^{13}C NMR (*DMSO*-d_6, δ, 50.3 MHz): 184.9 (C=O-7, 14), 168.1 (C-3, 4), 162.0 (C-1, 6), 158.3 (C-8, 13), 144.5 (C-10, 11), 128.2 (C-3a, 3b), 125.7 (C-6b, 14b), 122.9 (C-7c, 14c), 120.8 (C-10a, 10b), 117.8 (C-7b, 13b), 112.0 (C-9, 12), 110.9 (C-6a, 14a), 91.9 (C, 2, 5), 90.0 (C-7a, 13a), 23.3 (CH_3-10, 11) ppm; IR (KBr): $\nu = 3420$, 1638, 1619, 1580, 1461, 1367, 1239, 1204, 1132, 1040, 782 cm^{-1}; MS (*e*-spray ionization from water/acetonitrile = 1/1): $m/z = 822$ (2; M^+), 743 (5), 669 (2), 564 (4), 473 (6), 411 (17), 371 (20), 323 (19), 268 (100), 241 (45), 220 (35), 169 (6), 117 (13); UV/Vis (H_2O, $c = 7 \cdot 10^{-6}$ mol/l): $\lambda_{max} = 615$ (20800), 569 (8800), 470 (6000), 395 (14000), 340 (16800) nm (ε); fluorescence (H_2O, $c = 7 \cdot 10^{-6}$ mol/l): $\lambda_{em} = 625$ (1), 665 (0.3) nm (rel. intensity), $\Phi_{fluo} = 0.8$; UV/Vis (*DMSO*, $c = 8 \cdot 10^{-6}$ mol/l): $\lambda_{max} = 614$ (20800), 569 (8800), 470 (6000), 395 (14000), 340 (16800) nm (ε); UV/Vis (pyridine, $c = 1 \cdot 10^{-5}$ mol/l): $\lambda_{max} = 601$ (1), 569 (0.4), 470 (0.7) nm (rel. intensities); UV/Vis (acetone): $\lambda_{max} = 648$ nm; UV/Vis (ethanol): $\lambda_{max} = 645$ nm; UV/Vis (methanol): $\lambda_{max} = 625$, 450 nm; UV/Vis (acetonitrile): $\lambda_{max} = 645$ nm.

N-(2-Hydroxyethyl) acetamide

In analogy to Ref. [9], however, improved by changing equivalents, a mixture of 55 mg (0.9 mmol) of ethanolamine and 580 mg (2.5 mmol) of pentafluorophenyl acetate (prepared according to Ref. [9]) in 1.5 ml of dimethylformamide was allowed to stand overnight and was then evaporated to dryness under vacuum. The residue was dissolved in 15 ml of water, the excess of pentafluorophenyl acetate was separated, and the solution was evaporated and dried under vacuum again to get an advanced yield of 93 mg of N-(2-hydroxyethyl) acetamide (98%).

^{1}H NMR ($CDCl_3$, δ, 200 MHz): 6.15 (bs, 1H, -NH), 3.72 (t, $J = 4.8$ Hz, 2H, CH_2NH), 3.4 (q, 2H, CH_2OH), 2.01 (s, 1H, CH_3CO) ppm; ^{13}C NMR ($CDCl_3$, δ, 200 MHz): 171.6 (C=O), 61.9 (CH_2OH), 42.3 (CH_2NH), 23.0 (CH_3CO) ppm.

1,3,8-Tri-O-acetoxy-6-(ω-2-acetamino-ethoxy-methyl)-anthracene-9,10-dione (**5**; $C_{25}H_{23}NO_{10}$)

A mixture of 60 mg (0.11 mmol) 1,6,8-tri-O-acetoxy-3-iodomethyl-anthraquinone (prepared from emodin according to Ref. [7]), 120 mg (1.1 mmol) of N-(2-hydroxyethyl)-acetamide (prepared according to Ref. [9]), and 40 mg (0.15 mmol) of silver trifluoromethane sulfonate in 7 ml of CH_2Cl_2 was refluxed for 20 h. The reaction mixture was cooled to room temperature, diluted with 50 ml CH_2Cl_2, and filtered to remove AgI. The filtrate was washed 3 times with water, dried over Na_2SO_4, and evaporated. Column chromatography of the product mixture on silica using first a mixture of $CHCl_3$/EtOAc = 1/1 to remove the by-product, which was identified as 1,6,8-tri-O-acetoxy-3-hydroxymethyl-anthraquinone, and then $CHCl_3$/MeOH = 20/1 gave 20 mg of **3** (37%).

^{1}H NMR ($CDCl_3$, δ, 200 MHz): 8.14 (s, 1H, ar-H), 7.95 (d, $J = 2.5$ Hz, 1H, ar-H), 7.38 (s, 1H, ar-H), 7.24 (d, $J = 2.5$ Hz, 1H, ar-H), 6.0 (bs, 1H, NH-), 4.62 (s, 2H, ar-CH_2-O), 3.62 (t, $J = 4.8$ Hz, 2H, CH_2-NH), 3.53 (t, $J = 4.8$ Hz, 2H, CH_2-O), 2.44 (s, 6H, 2-CH_3COO), 2.35 (s, 3H, CH_3COO), 2.07 (s, 3H, CH_3CONH) ppm; ^{13}C NMR ($CDCl_3$, δ, 50.3 MHz): 181.1 (C=O of quinone), 179.5 (C=O of quinone), 170.3 (C=O of $NHCOCH_3$), 169.4 (C=O of CH_3COO), 169.0 (C=O of CH_3COO), 167.9 (C=O of CH_3COO), 154.7 (C_{ar}), 151.5 (C_{ar}), 150.4 (C_{ar}), 146.0 (C_{ar}), 135.5 (C_{ar}), 134.3 (CH_{ar}), 128.4 (CH_{ar}), 128.3 (C_{ar}), 124.5 (C_{ar}), 123.5 (CH_{ar}), 123.1 (C_{ar}), 118.3 (CH_{ar}), 71.3 (ar-CH_2-O), 69.9 (R-CH_2-O), 39.3 (CH_2-NH), 23.3 (CH_3 of $NHCOCH_3$), 21.1 (CH_3 of 3CH_3COO) ppm.

1,3,8-Trihydroxy-6-(ω-2-acetamino-ethoxy-methyl)-anthracene-9,10-dione (**6**; $C_{19}H_{17}NO_7$)

A solution of 55 mg (0.11 mmol) of **5** in 5 ml acetone and 2.5 ml of 3 *N* HCl was refluxed for 30 min; afterwards, another 2.5 ml of HCl were added and refluxed for further 2 h. Then the reaction mixture

was evaporated to remove acetone and extracted with ethylacetate. The organic phase was washed twice brine and water, dried over Na_2SO_4, and chromatographed on a silica column using a mixture of $CHCl_3$/MeOH = 10/1 as eluent to give 36 mg (90%) **6**.

^{1}H NMR (*DMSO*-d_6, δ, 200 MHz): 12.09 (s, 1H, OH), 12.08 (s, 1H, OH), 11.43 (s, 1H, OH), 8.03 (bt, 1H, NH), 7.62 (s, 1H, ar-H), 7.30 (s, 1H, ar-H), 7.14 (d, $J = 2$ Hz, ar-H), 6.61 (d, $J = 2$ Hz, ar-H), 4.59 (s, 2H, ar-CH_2-O), 3.48 (t, $J = 5.4$ Hz, 2H, CH_2-NH), 3.27 (t, $J = 5.4$ Hz, 2H, CH_2-O), 1.81 (s, 3H, CH_3CONH) ppm.

1,3,8-Trihydroxy-6-(ω-2-acetamino-ethoxy-methyl)-10H-anthracen-9-one (**7**; $C_{19}H_{19}NO_6$)

To a solution of 65 mg (0.17 mmol) of **6** in 16 ml of glacial acetic acid, 350 mg of $SnCl_2 \cdot 2H_2O$ (1.54 mmol) in 1.8 ml of conc. HCl were added under heating and refluxed for additional 10 min. After cooling, the reaction mixture was poured into 150 ml of cold brine. The precipitate was centrifuged, washed with water, and dried under vacuum at 60°C to yield 55 mg (90%) **7**.

^{1}H NMR (*DMSO*-d_6, 200 MHz): 12.32 (s, 1H, OH), 12.36 (s, 1H, OH), 10.87 (s, 1H, OH), 8.0 (bs, 1H, NH), 6.88 (s, 1H, ar-H), 6.79 (s, 1H, ar-H), 6.41 (s, 1H, ar-H), 6.22 (d, $J = 1.8$ Hz, ar-H), 4.48 (s, 2H, ar-CH_2-O), 4.31 (s, 2H, ar-CH_2-ar), 3.45 (t, $J = 5.6$ Hz, 2H, CH_2-NH), 3.26 (t, $J = 5.5$ Hz, 2H, CH_2-O), 1.81 (s, 3H, CH_3CONH) ppm; ^{13}C NMR ($CDCl_3$, δ, 50.3 MHz): 191.1 (C=O quinone), 169.3 (C=O of $NHCOCH_3$), 165.1 (C_{ar}), 164.6 (C_{ar}), 161.7 (C_{ar}), 147.3 (C_{ar}), 145.0 (C_{ar}), 142.1 (C_{ar}), 117.1 (CH_{ar}), 113.9 (C_{ar}), 112.7 (CH_{ar}), 108.4 (C_{ar}), 107.4 (CH_{ar}), 101.1 (CH_{ar}), 70.9 (ar-CH_2-O), 68.9 (O-CH_2), 39.0 (overlap with *DMSO* peak, CH_2-NH), 32.4 (ar-CH_2-ar), 22.5 (CH_3CONH) ppm.

1,3,4,6,8,13-Hexahydroxy-10,11-bis-(ω-2-acetamino-ethoxy-methyl)-phenanthro[1,10,9,8-o,p,q,r,a]-perylene-7,14-dione (**8**; $C_{38}H_{30}N_2O_{12}$)

A mixture of 80 mg (0.22 mmol) anthrone **7**, 130 mg (1.44 mmol) pyridine-N-oxide, 100 µl piperidine, and 9 mg $FeSO_4 \cdot 7H_2O$ dissolved in 1 ml of pyridine was stirred in the dark at 110°C for 1.5 h, cooled to room temperature, and quenched with a solution of 1.5 ml conc. HCl in 15 ml H_2O. After standing for 1 h, the precipitate was isolated by centrifugation and washed once with water. The precipitate was suspended in 600 ml of acetone and irradiated with a 500 W photo flood lamp for 1.5 h. The resulting suspension was filtered and the filtrate was evaporated. Preparative silica thin layer chromatography of the residue using a mixture of $CHCl_3$/MeOH = 4/1 as the eluent gave 9 mg (10%) **8**.

M.p.: not below 300°C; ^{1}H NMR (acetone-d_6, δ, 200 MHz): 18.84 (bs, OH-3), 14.83 and 14.27 (2s, OH-1, 6 and OH-8, 13), 7.66 (s, H-9, 12), 7.08 (bs, 2NH), 6.62 (s, H-2, 5), 5.16 and 4.70 (AB-system, $J = 12$ Hz, ar-CH_2O), 3.5 (m, $2CH_2$N), 3.2 (m, $2CH_2$O), 1.76 (s, $2CH_3$) ppm; UV/Vis (ethanol, $6 \cdot 10^{-6}$ mol/l): $\lambda_{max} = 594$ (43333), 550 (20666), 512 (7333), 480 (10666), 386 (10000), 328 (26500), 286 (39300) nm (ε).

1,3,4,6,8,13-Hexahydroxy-10,11-bis-(ω-2-amino-ethoxy-methyl)-phenanthro[1,10,9,8-o,p,q,r,a]perylene-7,14-dione (**9**; $C_{34}H_{26}N_2O_{10}$)

The acetamino derivative **8** could be saponified by means of boiling 6 *N* HCl; however, neither the obtained hydrochloride nor the free base could be dissolved in any solvent. IR (KBr): $\nu = 3448$, 2923, 1617, 1465, 1247, 1112, 951 cm^{-1}.

1,3,8-Trihydroxy-6-(ω-2-chloro-ethoxy-methyl)-anthracene-9,10-dione (**10**; $C_{17}H_{13}O_6Cl$)

A mixture of 160 mg (0.33 mmol) 1,6,8-tri-O-acetoxy-3-bromomethyl-anthraquinone (prepared from emodin according to Ref. [6]), 4.5 g (55 mmol) of 2-chloroethanol, and 150 mg (0.58 mmol) $AgClO_4$

was heated at 90°C for 3 h. the reaction mixture was cooled to room temperature, diluted with 150 ml CH_2Cl_2, and filtered. The filtrate was washed with brine and H_2O, dried over Na_2SO_4, and evaporated. Column chromatography of the product mixture on silica using a mixture of $CHCl_3$/MeOH = 20/1 gave 110 mg of **10** (95%).

^{1}H NMR (*DMSO*-d_6, δ, 200 MHz): 12.08 (bs, 2H, 2OH), 11.45 (bs, 1H, OH), 7.65 (s, 1H, ar-H), 7.29 (s, 1H, ar-H), 7.14 (d, $J = 2.3$ Hz, 1H, ar-H), 6.62 (d, $J = 2.3$ Hz, 1H, ar-H), 4.68 (s, 2H, ar-CH_2-O), 3.89–3.76 (m, 4H, CH_2-O and CH_2-Cl) ppm; ^{1}H NMR (acetone-d_6, δ, 200 MHz): 12.16 (s, 1H, OH), 12.13 (s, 1H, OH), 10.19 (bs, 1H, OH), 7.72 (s, 1H, ar-H), 7.32 (s, 1H, ar-H), 7.27 (d, $J = 2.3$ Hz, 1H, ar-H), 6.68 (d, $J = 2.3$ Hz, 1H, ar-H), 4.76 (s, 2H, ar-CH_2-O), 389–3.76 (m, 4H, CH_2-O and CH_2-Cl) ppm; ^{13}C NMR (*DMSO*-d_6, δ, 50.3 MHz): 190.5 (C=O), 182.1 (C=O), 166.5 (C_{ar}), 165.4 (C_{ar}), 162.2 (C_{ar}), 149.1 (C_{ar}), 135.9 (C_{ar}), 134.0 (C_{ar}), 122.5 (CH_{ar}), 118.4 (CH_{ar}), 115.5 (C_{ar}), 109.3 (C_{ar}), 109.7 (CH_{ar}), 108.3 (CH_{ar}), 71.3 (ar-CH_2-O), 71.1 (R-CH_2-O), 44.5 (CH_2-Cl) ppm.

1,3,8-Trihydroxy-6-(ω-2-chloro-ethoxy-methyl)-10H-anthracen-9-one (**11**; $C_{17}H_{15}O_5Cl$)

To a solution of 110 mg (0.31 mmol) of **10** in 24 ml glacial acetic acid, 400 mg of $SnCl_2 \cdot 2H_2O$ (1.76 mmol) dissolved in 2.8 ml of conc. HCl were added under heating and refluxed for additional 10 min. After cooling, the reaction mixture was poured into 150 ml of cooled brine. The precipitate was centrifuged, washed with water, and dried under vacuum at 60°C to yield 93 mg (90%) of **11**.

^{1}H NMR (acetone-d_6, δ, 200 MHz): 12.47 (s, 1H, OH), 12.37 (s, 1H, OH), 9.8 (bs, 1H, OH), 6.99 (s, 1H, ar-H), 6.88 (s, 1H, ar-H), 6.55 (s, 1H, ar-H), 6.35 (d, $J = 1.8$ Hz, ar-H), 4.66 (s, 2H, ar-CH_2-O), 4.38 (s, 2H, ar-CH_2-ar), 3.83–3.76 (m, 4H, CH_2-O and CH_2-Cl) ppm; ^{1}H NMR (*DMSO*-d_6, δ, 200 MHz): 12.37 (s, 1H, OH), 12.31 (s, 1H, OH), 10.9 (bs, 1H, OH), 6.94 (s, 1H, ar-H), 6.83 (s, 1H, ar-H), 6.45 (s, 1H, ar-H), 6.25 (d, $J = 2.2$ Hz, ar-H), 4.66 (s, 2H, ar-CH_2-O), 4.38 (s, 2H, ar-CH_2-ar), 3.83–3.76 (m, 4H, CH_2-O and CH_2-Cl) ppm.

1,3,4,6,8,13-Hexahydroxy-10,11-bis-(ω-2-piperidinyl-ethoxy-methyl)-phenanthro-[1,10,9,8-o,p,q,r,a]perylene-7,14-dione (**12**; $C_{44}H_{42}O_{10}N_2$)

A mixture of 115 mg (0.34 mmol) **11**, 200 mg (2.2 mmol) pyridine-N-oxide, 200 µl piperidine, and 12 mg $FeSO_4 \cdot 7H_2O$ dissolved in 2 ml of pyridine was stirred in the dark at 110°C for 1 h, cooled to room temperature, and quenched with a solution of 2 ml conc. HCl in 20 ml H_2O. After standing for 1 h, the precipitate was isolated by centrifugation and washed once with water. The precipitate was suspended in 1000 ml acetone and irradiated with a 500 W photo flood lamp for 1.5 h. The resulting suspension was filtered and the filtrate was evaporated. Column chromatography of the residue on Sephadex LH-20 using MeOH as the eluent gave 10 mg (7%) of **11**.

^{1}H NMR (*DMSO*-d_6, δ, 200 MHz): 14.83 and 14.32 (2s, OH-1, 6 and OH-8, 13), 7.52 (s, H-9, 12), 6.53 (s, H-2, 5), 5.1 and 4.6 (AB-system, $J = 12$ Hz, ar-CH_2O), 3.5 (m, 4H, 2CH_2N), 3.3 (m, 4H, 2CH_2O), 2.8 (m, 8H, NCH_2 of piperidinyl group), 1.5 (m, 12H, CH_2 of piperidinyl group) ppm; ^{13}C NMR (*DMSO*-d_6, 50.3 MHz): 184.5 (C=O), 176.1 (C_{ar}), 169.1 (C_{ar}), 162.3 (C_{ar}), 145.1 (C_{ar}), 128.2 (C_{ar}), 122.3 (C_{ar}), 120.2 (C_{ar}), 119.8 (CH_{ar}), 118.5 (CH_{ar}), 110.8 (C_{ar}), 106.2 (CH_{ar}), 102.8 (CH_{ar}), 72.5 (ar-CH_2-O), (R-CH_2-O), 71.4 (R-CH_2-O), 50.9 (CH_2N of piperidinyl), 42 (overlap with *DMSO* peak; CH_2N), 25.1, 23.4 (CH_2 of piperidinyl) ppm; UV/Vis (ethanol, $6 \cdot 10^{-6}$ mol/l): $\lambda = 591$ (41130), 547 (20833), 510 (9167), 474 (12983), 380 (11600), 327 (29620) nm (ε); UV/Vis (*DMSO*, $6 \cdot 10^{-6}$ mol/l): $\lambda = 601$ (43300), 556 (22275), 517 (13500), 485 (13562), 388 (17125), 330 (32550) nm (ε); UV/Vis (acetone, $6l \cdot 10^{-6}$ mol/l): $\lambda = 598$ (43625), 553 (21125), 512 (4762), 460 (7975), 382 (6137) nm (ε).

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