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A Route to Amino Functionalized Hypericin Derivatives and their Chemical and Photochemical Properties Pertaining to Photodynamic Therapy

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Summary. Syntheses of amino functionalized hypericin derivatives could be achieved starting from the recently prepared emodin derived 1,3,8-trimethoxy-6-amino-9,10-anthraquinone. Our strategies for the preparation of 10,11-didemethyl-10,11-diaminohypericin, 10,11-didemethyl-10,11-di(acetylamino)-hypericin, and its hypericinoidic diazepine derivative include synthetical modifications on the levels of the anthraquinone, anthron, and the phenanthroperylenequinone system itself. The chemical as well as photochemical properties of these unique hypericin derivatives, which might constitute new photodynamic therapy agents, are reported.

Keywords. Anthraquinones; Anthrons; Microwave assisted synthesis; Phenanthroperylenequinones; Seven-membered ring closure.

Introduction

The naturally occurring polyhydroxylated phenanthroperylenequinone, hypericin (**1**), represents one of the most powerful photosensitizing compounds found in nature [1, 2] and therefore finds application in the photodynamic therapy (PDT) of cancer (see Fig. 1). In addition, **1** shows broad anti-cancer and anti-viral activity [2], which makes it extraordinarily interesting for a variety of biological and medical applications. Unfortunately, this natural photosensitizer possesses two main disadvantages concerning its application in PDT, which are a very limited solubility under physiological conditions as well as a long-wavelength absorption ($\lambda_{max} \leq 600$ nm), which is below the emission lines of most lasers used in medical applications. We therefore

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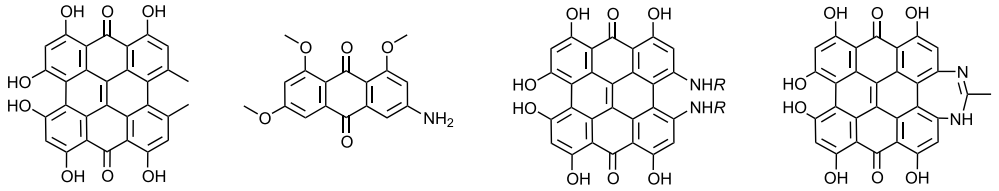


Fig. 1. Structures of hypericin (**1**), tri-*O*-methyl emodic amine (**2**), 10,11-didemethyl-10,11-diamino-hypericin (**3**), 10,11-didemethyl-10,11-di(acetylamino)hypericin (**4**), and the hypericinoidic diazepine derivative **5**

focused on the synthetic modification of **1** aiming to leave the core phenanthroperylenequinone system untouched and to achieve improved solubility and absorption characteristics. Of course, these modifications should simultaneously maintain the ability of singlet oxygen and/or reactive oxygen species generation comparable to, or even better than for the natural product **1**. Recently, a series of heterocyclically appended nitrogen containing hypericin derivatives have been synthesized [3, 4], which have been found to display an enhanced solubility, a pronounced bathochromic shift of the long-wavelength absorption band, and the ability to generate singlet oxygen and/or reactive oxygen species comparable to **1**.

Following our quest for such second generation photosensitizers we targeted the synthesis of a new class of amino functionalized photodynamically active hypericin derivatives starting from the recently synthesized 1,3,8-trimethoxy-6-amino-9,10-anthraquinone (**2**, tri-*O*-methyl emodic amine) [5] (see Fig. 1). Herein, we report our efforts to synthesize the amino substituted hypericin derivatives **3**, **4**, and **5** as well as the investigations of their chemical and photochemical properties pertaining to PDT.

Results and Discussion

Syntheses of Amino Functionalized Hypericin Derivatives

The retrosynthetical approach for the synthesis of 10,11-didemethyl-10,11-diaminohypericin (**3**) and its derivatives initially followed the already well established

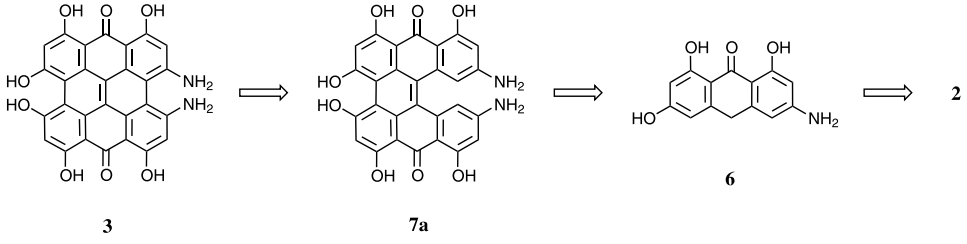


Fig. 2. Retrosynthetical approach for the synthesis of 10,11-didemethyl-10,11-diaminohypericin (**3**) starting from the aminoanthraquinone **2**

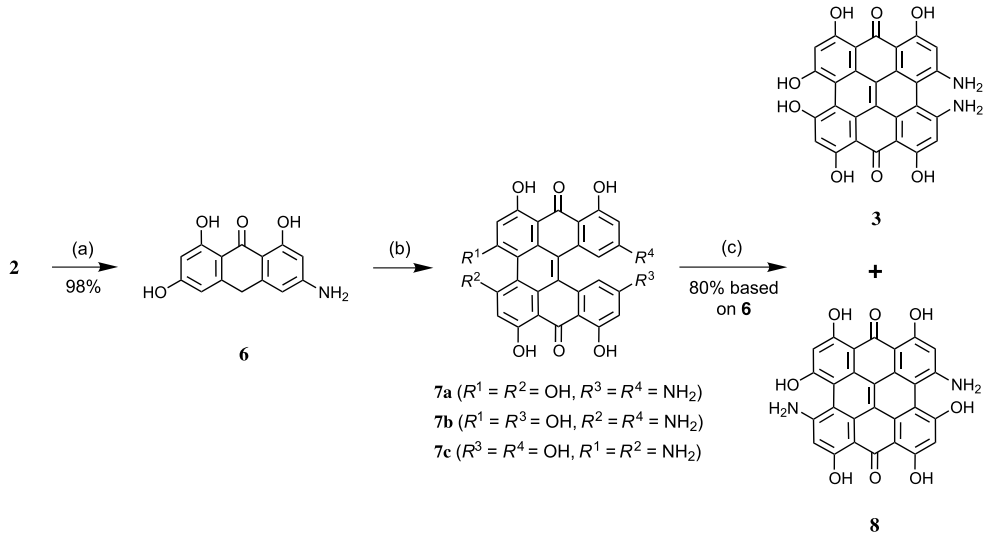


Fig. 3. Deprotection/reduction of tri-*O*-methyl emodic amine (**2**) to the corresponding anthron **6** and its unexpected unselective coupling, which ends up with an isomeric mixture of **7a**–**7c**, followed by the photocyclization to the target 10,11-didemethyl-10,11-diaminohypericin (**3**) and its isohypericinoidic analogue **8**; (a) HO*Ac*/$SnCl_2 \cdot 2H_2O$/HBr, 105°C, 30 min; (b) $FeSO_4 \cdot 7H_2O$/pyridine-*N*-oxide/pyridine/piperidine, 115°C, 1 h, light protection; (c) *Me*OH, $h\nu$, 2 h

three-step sequence [1, 6–7] for hypericin syntheses starting from the anthraquinone system (see Fig. 2). Thus, subsequent deprotection/reduction of tri-*O*-methyl emodic amine (**2**) should provide the corresponding aminoanthron **6**. This could be dimerized to the protohypericin derivative **7a,** and eventually photocyclized to the primary diaminohypericin target **3**.

The conversion of the recently synthesized synthon tri-*O*-methyl emodic amine (**2**) [5] to the corresponding 1,3,8-trihydroxy-6-amino-10*H*-anthracen-9-one (**6**) was performed in analogy to Ref. [6] using optimized conditions, which became necessary specifically for this amino derivative. Accordingly, reduction/deprotection of **2** to the corresponding anthron **6** by heating a mixture of **2**, $SnCl_2 \cdot 2H_2O$, and HBr in glacial acetic acid at 105°C for 30 min under Ar provided **6** in 98% yield (see Fig. 3). Dimerization of **6** was carried out in the conventional way [7] using a stirred solution of **6**, $FeSO_4 \cdot 7H_2O$, pyridine-*N*-oxide, piperidine, and pyridine under Ar and protection from light. Finally, heating at 115°C for 1 h should have provided us with the light sensitive protohypericin derivative **7a**. Surprisingly however, we obtained an isomeric mixture of the three constitutional isomers **7a**–**7c**, which afforded after the photocyclization step a 1:1 mixture of the desired 10,11-didemethyl-10,11-diaminohypericin (**3**), but also its isohypericinoidic constitutional isomer **8** in 80% overall yield based on **6**. By using ^{1}H NMR and mass spectrometry it could be unequivocally established, that after the photocyclization step a mixture of the two constitutional isomers **3** and **8** was obtained indeed.

The reason for this unselective coupling observed for the first time in the case of **6** might be caused by the similar activation of position 4 by the 3-OH group and of position 5 by the 6-NH_2 group of the

anthron **6**. Therefore, the dimerization step resulted in an unselective reaction yielding the three possible constitutional isomers **7a**–**7c**. Up to this case, all modifications of the anthron system in the synthesis of novel hypericin derivatives did not change the activation/deactivation behaviour and therefore the coupling did take place regioselectively.

The separation of the hypericinoidic and isohypericinoidic compounds **3** and **8** by column as well as preparative thin layer chromatography could not be achieved. It was not unexpected that the two isomers **3** and **8** showed similar chromatographic behaviour, which was due to their negligible differences concerning polarity and chemical properties. Furthermore, derivatization reactions to differentiate the two isomers, like *e.g.* the condensation reaction between diamines and 1,2-dicarbonyl compounds in analogy to Ref. [8], were unsuccessful.

Since all efforts for a separation of **3** and **8** failed, we decided to change our strategy for synthesizing **3** or its derivatives directly on the level of the anthraquinone derivative **2**. Thus, we thought to modify the highly activating free amino group into a deactivating one to achieve a selective coupling reaction. Useful amino protecting groups must therefore be stable under acidic conditions, which is indispensable with respect to the deprotection/reduction step under acidic conditions to the corresponding anthron. For this purpose only the acid stable *Fmoc*-protecting group [9] seemed feasible. It should be cleaved very fast under certain basic conditions. However, since piperidine is one of the most common bases for the cleavage of *Fmoc*-groups, this protecting group became also unsuitable, because piperidine acts as a reagent in the dimerization step of the anthron to the protohypericin derivative. In this case the deprotection of the *Fmoc*-group would proceed faster than the dimerization and we would end up with the same problem of unselective coupling. Furthermore, the use of photolabile protecting groups like *e.g.* 6-nitroveratryloxycarbonyl was also put aside because of their acid lability similar to those of the benzyloxycarbonyl protecting group [9].

Changing our strategy for a selective coupling we eventually converted the tri-*O*-methyl emodic amine (**2**) into the 1,3,8-trimethoxy-6-(acetylamino)-9,10-anthraquinone (**9**). Thus, N-acetylation of **2** to the corresponding acetamide **9** by refluxing a mixture of **2**, acetyl chloride, and pyridine in 1,4-dioxan in analogy to Ref. [10] for 15 min under Ar provided **9** in 98% yield (see Fig. 4). Subsequent deprotection/reduction of the acetamide **9** was performed according to the above mentioned procedure for the synthesis of **6**. Thus, heating a mixture of **9**, $SnCl_2 \cdot 2H_2O$, and HBr in glacial acetic acid at 105°C for 30 min under Ar did not

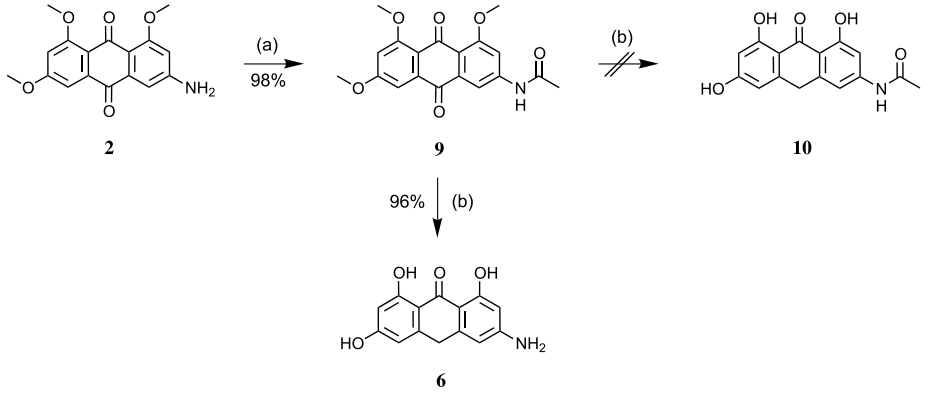


Fig. 4. N-Acetylation of tri-*O*-methyl emodic amine (**2**) to 1,3,8-trimethoxy-6-(acetylamino)-9,10-anthraquinone (**9**) and its subsequently failed deprotection/reduction to the target (acetylamino)-anthron **10** which ended up in the anthron **6** quantitatively; (a) 1,4-dioxan/acetyl chloride/pyridine, reflux, 15 min; (b) HO*Ac*/$SnCl_2 \cdot 2H_2O$/HBr, 105°C, 30 min

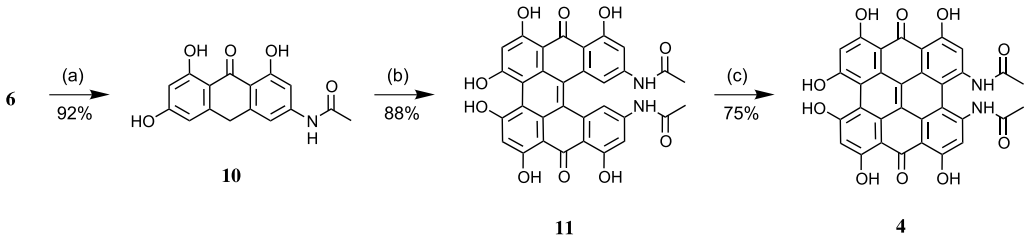


Fig. 5. Selective N-acetylation of 1,3,8-trihydroxy-6-amino-10*H*-anthracen-9-one (**6**) to **10** and its subsequent selective dimerization to 10,11-didemethyl-10,11-di(acetylamino)protohypericin (**11**) followed by photocyclization to the corresponding 10,11-didemethyl-10,11-di(acetylamino)-hypericin (**4**); (a) 1,4-dioxan/acetyl chloride/pyridine, reflux, 1 h; (b) $FeSO_4 \cdot 7H_2O$/pyridine-*N*-oxide/pyridine/piperidine, 115°C, 1 h, light protection; (c) acetone, $h\nu$, 1.5 h

provide the (acetylamino)anthron **10**, but the fully deprotected anthron **6** in 98% yield (see Fig. 4).

At this point it should be mentioned, that the selective cleavage of the amide bond might happen only at the stage of the anthron system. We will show later on, that under the deprotection/reduction conditions used (Sn(II)/HO*Ac*/HBr), the amide bond will be perfectly stable as long as it is at the level of a quinoid system, like *e.g.* in the case of the 10,11-didemethyl-10,11-di(acetylamino)hypericin (**4**).

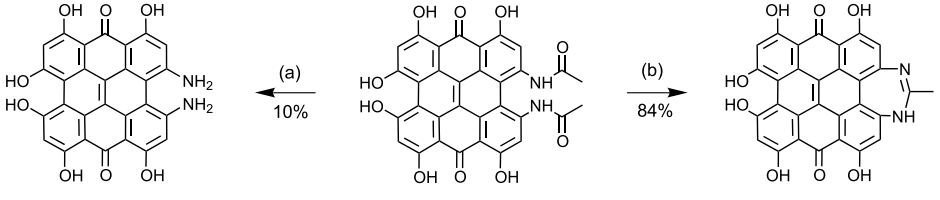
According to these results, we tried to modify the amino group and consequently its electronic effect directly on the level of the anthron itself. Of course, this bears the problem of free phenolic hydroxyl groups on the one hand, and the generally known extraordinary instability of most of the anthrons in solution on the other hand. However, refluxing a mixture of **6**, acetyl chloride, and pyridine in 1,4-dioxan for 1 h under Ar provided the (acetylamino)anthron **10** selectively in 92% yield (see Fig. 5). Due to the fact that the acetylamino group of **10** exerts a more deactivating effect in comparison to the free amino group we thought to be able to selectively dimerize **10** to the corresponding protohypericin **11**. Thus, dimerization of **10** was carried out using $FeSO_4 \cdot 7H_2O$, pyridine-*N*-oxide, piperidine, and pyridine under Ar and protection from light [7]. Heating at 115°C for 1 h regioselectively yielded 88% of the light sensitive protohypericin derivative **11**. Cyclization of **11** by irradiation afforded the desired hypericin derivative **4**, which was isolated in 75% yield (61% overall yield based on **6**, Fig. 5). Our recently developed synthesis of the anthraquinone synthon **2** for this sequence proceeded in 79% overall yield [5] starting from the readily available natural product emodin (**12**, 1,3,8-trihydroxy-6-methyl-9,10-anthraquinone). Therefore we were able to synthesize this unique di(acetylamino)hypericin derivative **4** in eight steps with an overall yield of 48% starting from **12**.

Beside the fact that 10,11-didemethyl-10,11-di(acetylamino)hypericin (**4**) represents an interesting amino functionalized hypericin derivative itself, **4** seemed to be also a potential starting material for the synthesis of the corresponding diaminohypericin analog **3**. However, the cleavage of the two amide bonds of **4** turned out to be more difficult than expected. Using the system HO*Ac*/HBr under reflux conditions only **4** was recovered unchanged. Furthermore, the conversion of

4 to **3** failed even under reductive deprotection conditions using the system $SnCl_2 \cdot H_2O/HOAc/HBr$. Amide bond cleavage did not take place at all and even after 2 h under harsh reflux conditions the di(acetylamino) derivative **4** was recovered unchanged. This result strengthened our opinion (*cf.* above), that an amide bond cleavage during the conversion of the anthraquinone **9** to the anthron **6** exclusively took place at the level of an anthron system but not on the quinoid system itself. Application of basic deprotection methods like *e.g.* the system hydrazine hydrate/*Me*OH [11] under reflux for 30 min was not successful and led to recovery of unchanged **4** only. By using *N*,*N*,*N*′,*N*′-tetramethylguanidine (*TMG*) in *Me*OH [11] under reflux conditions deprotection to **3** occurred, whereas under milder reaction conditions **4** was recovered unchanged. However, this deprotection under elevated temperature was accompanied by severe decomposition and afforded **3** in unsatisfying amounts. The application of the system $KOH/MeOH/H_2O$ [12] at 80°C for 2.5 h ended up in a mixture of the starting material **4** and its partially and fully deprotected derivatives. Even by prolonging the reaction time (>2.5 h) a full deprotection of **4** to **3** was not possible, but ended up with an increased amount of decomposition products like in the case of *TMG*. Nevertheless, the isolation of **3** was possible by using the system $KOH/MeOH/H_2O$ after purification in yields <10% (see Fig. 6). Up to now, we were not able to synthesize the diaminohypericin analog **3** by a selective, high yield protocol, but only in amounts sufficient to perform a limited characterization.

Following our desire for the synthesis of the hypericinoidic diazepine derivative **5** we started with the di(acetylamino)hypericin derivative **4**. Performing a seven-membered intramolecular ring closure strategy pyridinium chloride was found to be an extraordinarily suitable new deacetylation/dehydration reagent. Different reagents for this purpose are known [13], *e.g.* in the synthesis of 2-substituted benzimidazoles starting either from the corresponding 1,2-diamino-, 1-amino-2-(acetylamino)-, or 1,2-di(acetylamino) derivatives, but to the best of our knowledge, pyridinium chloride has not been used at all.

Deacetylation/dehydration of 10,11-didemethyl-10,11-di(acetylamino)hypericin (**4**) was carried out by using microwave assisted synthesis by irradiating a mixture of **4** and pyridinium chloride at 400 W for 2 min under Ar and reflux conditions (223°C). This afforded the desired hypericinoidic diazepine derivative



3 **4** **5**

Fig. 6. Deprotection of 10,11-didemethyl-10,11-di(acetylamino)hypericin (**4**) to **3** and the conversion of **4** to the hypericinoidic diazepine derivative **5** by means of an intramolecular seven-membered ring closure strategy; (a) $KOH/MeOH/H_2O$, 80°C, ~2.5 h; (b) *Method A*: pyridinium chloride, MW (400 W), reflux, 2 min; *Method B*: pyridinium chloride, 150°C, 6 h

5 in 82% yield (see Fig. 6). It should be mentioned that the same result could be achieved by conventional synthesis by melting a mixture of **4** and pyridinium chloride for 6 h at 150°C under Ar. Thus, we were able to synthesize this unique hypericinoidic diazepine derivative **5** in nine steps with an overall yield of 39% starting from the natural product emodin (**12**).

It is noteworthy that beside the abovementioned attempts for the di-N-deacetylation of **4** to the didemethyldiaminohypericin derivative **3** two further reagent combinations, which have been primarily used in the *O*-methyl ether cleavage were tested. Both, the systems $AlCl_3/NaCl$ [14] as well as KI/H_3PO_4 [15] were used for this amide bond cleavage under rather harsh conditions (>150°C). However, by means of mass spectrometry it could be unequivocally established that di-N-deacetylation of **4** to **3** did not take place at all, but deacetylation/dehydration to the seven-membered diazepine derivative **5** occurred also under these conditions.

Chemical and Photochemical Properties of Amino Functionalized Hypericin Derivatives

As mentioned above, the natural photosensitizer hypericin (**1**) possesses two main disadvantages concerning its applicability in PDT, which are a very limited solubility under physiological conditions as well as a long-wavelength absorption ($\lambda_{max} \sim 600$ nm), which is below the emission lines of most lasers used in medical applications. Synthetic modification of **1** should thus lead to better solubility and absorption characteristics and maintain simultaneously the ability of singlet oxygen and/or reactive oxygen species generation of **1** or even improve it. Thus, we investigated the chemical and photochemical behaviour of the novel amino functionalized hypericin derivatives **3**, **4**, and **5** concerning their applicability in PDT.

With respect to **3** the small quantity obtained in the synthesis efforts proved to be insufficient to evaluate its photochemical properties in detail. However, it can be stated that substitution of the two methyl groups of **1** by two amino groups to yield **3** caused a bathochromic shift of nearly 20 nm. Fortunately, a satisfying bathochromic shift of the long-wavelength absorption of $\Delta\lambda_{max} \sim 10$–$14$ nm (in a series of solvents) was also observed for **4** ($\lambda_{max} \sim 602$–616 nm) in comparison to hypericin (**1**, $\lambda_{max} \sim 592$–602 nm). In contrast to the di(acetylamino) derivative **4**, the hypericinoidic diazepine derivative **5** showed a less bathochromically shifted absorption band in the range of $\lambda \sim 595$–609 nm ($\Delta\lambda \sim 3$–5 nm), but in addition to this band a long-wavelength absorption band at $\lambda_{max} \sim 675$–685 nm was observed for solutions in *DMF*, *DMSO*, pyridine, and *THF*. Thus, one of the main targets, which is the shifting of the long-wavelength absorption band of hypericin (**1**) towards the emission wavelength of medical lasers, was achieved by the syntheses of **4** and **5**. Regarding the solubility of these amino functionalized hypericin derivatives it should be stressed that the di(acetylamino) analog **4** is extraordinarily soluble ($>2\ mg \cdot cm^{-3}$) in all common polar and nonpolar organic solvents (acetone, acetonitrile, *DMF*, *DMSO*, 80% aqueous ethanol, ethylacetate, methanol, pyridine, and *THF*) thus, solubility being significantly better than the one of hypericin (**1**), which is of particular interest for a proper solubility under physiological conditions. The molar extinction coefficients of the long-wavelength absorption band of **4** in

these solvents are in the range of $\varepsilon \sim 13000–17000\,dm^3 \cdot mol^{-1} \cdot cm^{-1}$, which amounts approximately to one third of that observed for hypericin (**1**) itself [1]. The solubility behaviour of the hypericinoidic diazepine derivative **5** significantly differs from that of the di(acetylamino) derivative **4** and even from **1**. Thus, **5** is only soluble ($<1\,mg \cdot cm^{-3}$) in *DMF*, *DMSO*, pyridine, and *THF*, whereas the solubility in acetone, acetonitrile, and ethanol is only in the range of $\sim 0.1\,mg \cdot cm^{-3}$. Its insolubility in solvents like *e.g.* methanol, ethylacetate, and 80% aqueous ethanol in particular is unsatisfying from that point of view. However, by means of small amounts of *DMSO* (~5%) added as a solution mediator, **5** displays also a proper solubility in these solvents. The molar extinction coefficients of the long-wavelength absorption band of **5** in acetone, acetonitrile, and ethanol are comparable to that observed for **4**. In *DMF*, *DMSO*, pyridine, and *THF* the molar extinction coefficients of the band at ~600 nm of **5** are in the range of $\varepsilon \sim 20000–39000\,dm^3 \cdot mol^{-1} \cdot cm^{-1}$ which amounts to more than half of that observed for hypericin (**1**) [1]. In addition, the hypericinoidic diazepine derivative **5** shows a molar extinction coefficient of $\varepsilon = 1000–4000\,dm^3 \cdot mol^{-1} \cdot cm^{-1}$ for its long-wavelength absorption band ($\lambda_{max} \sim 675–685$ nm) in *DMF*, *DMSO*, pyridine, and *THF* solutions.

The spectrophotometric titration [16] of **4** in 80% aqueous ethanol as well as **5** in 80% aqueous *DMSO* provided information concerning their protonation and deprotonation behaviour. In general, both amino functionalized hypericin derivatives showed acid/base characteristics, which are comparable to those of hypericin (**1**) itself. It became evident that, depending on the *pH*-values of the 80% aqueous ethanol or *DMSO* solutions, a certain species dominated below $pH \sim 1$, representing the monoprotonated form $\mathbf{4} \cdot H^+$ or $\mathbf{5} \cdot H^+$. Another species prevailed within the range of $pH = 1–3$ originating from the neutral form of **4** or **5** followed by a third species predominant above $pH \sim 3$, representing the monodeprotonated form $\mathbf{4}^{(-)}$ or $\mathbf{5}^{(-)}$. Finally, a fourth species became prominent above $pH \sim 12.5$ originating from the dideprotonated form $\mathbf{4}^{(2-)}$ or $\mathbf{5}^{(2-)}$. Since the *bay*-hydroxyl groups of hypericin (**1**) are acidic ($pK_a \sim 2$ [16]), it is obvious that the basic nitrogen of the hypericinoidic diazepine derivative **5** might form a zwitterion due to protonation of the diazepine nitrogen by its acidic *bay*-hydroxyl proton. Of course, it was not possible to find evidence for a zwitterion of **4** because of the extremely low basicity of the amides as compared to the pyridinic nitrogen atom of **5**. It should be stressed that the strong acidity of the *bay*-phenolic proton observed in the titration experiments is in agreement with the hypericinoidic and not the isohypericinoidic [16, 17] constitution of **4** and **5**.

The structures of 10,11-didemethyl-10,11-di(acetylamino)hypericin (**4**) and its corresponding hypericinoidic diazepine derivative **5** were assigned from their characteristic IR absorption bands and mass spectra. In addition, a full structure elucidation of the hypericin derivative **4** was performed by NMR spectroscopy (^1H and ^{13}C NMR), in particular by advanced 2D NMR measurements including HSQC, HMBC, and N*O*ESY experiments. It is noteworthy, that also the precursor of **4**, the corresponding light-sensitive 10,13-didemethyl-10,13-di(acetylamino)-protohypericin (**11**) could be fully elucidated by means of 1D and 2D NMR experiments. To the best of our knowledge, this full structural assignment of a protohypericin derivative is unique in literature. It should be mentioned, that we

were thus able to find direct evidence for our proposed C_2-symmetrical structure of the protohypericin derivative **11**. Thus, the ^{1}H NMR spectrum of **11** showed three aromatic signals in the range of 6.3–7.5 ppm with an integral value of two protons each, representing ar-H2/H5, ar-H9/H14, and ar-H11/H12. In addition, we observed a HMBC correlation between the C9/C14 and C11/C12 to the amidic proton of 10/13-N*H*COCH$_3$, and accordingly, these correlations corroborated definitely the proposed C_2-symmetrically structured 10,13-didemethyl-10,13-di(acetylamino)protohypericin (**11**). In the case of an unsymetrically dimerized di(acetylamino)protohypericin derivative we would have expected to find six inequivalent aromatic protons as well as inequivalent signals for the –NH and –OH protons. Finally, the hypericinoidic diazepine derivative **5** could be structurally characterized by its ^{1}H NMR spectrum and its IR and mass data. In particular, its mass spectrometric fragmentation pattern corroborated the assigned structure. Its ^{13}C NMR spectrum could not be recorded due to its already mentioned poor solubility.

With respect to conformational details of the aminohypericin derivatives **3** and **4** and the diazepine **5** rough MMFF94–AM1 calculations [18] corroborated the high stabilities of the 7,14-dioxo tautomers observed for **1** [1] and also showed that the helical deformations in the 10,11-*bay* regions are slightly smaller than the ones observed for hypericin (**1**, $\theta_{3,3a,3b,4} = 14.3°$, $\theta_{10,10a,10b,11} = 28.3°$) [1]. Thus, the amino substituents of **3** ($\theta_{3,3a,3b,4} = 14.5°$, $\theta_{10,10a,10b,11} = 21.8°$) and **4** ($\theta_{3,3a,3b,4} = 14.3°$, $\theta_{10,10a,10b,11} = 24.1°$) are slightly less space demanding than the methyl groups of **1**, but obviously the torsional deformation of the skeleton is mainly due to the interactions of the *bay*-carbon atoms. The helical deformation of the skeleton is somewhat reduced by the conformational stress of the fused seven-membered ring of the azepine in **5** ($\theta_{3,3a,3b,4} = 14.7°$, $\theta_{10,10a,10b,11} = 16.5°$).

Recently synthesized nitrogen containing heterocyclically appended hypericin derivatives [3, 4] have shown the ability to generate singlet oxygen and/or reactive oxygen species to a somewhat lesser or otherwise even higher extent as hypericin (**1**) itself. In contrast to that there are also nitrogen containing hypericin derivatives [6], which have not displayed any photosensitizing ability at all. However, the 10,11-didemethyl-10,11-di(acetylamino)hypericin (**4**) now proved to be quite effective in the sensitized destruction of bilirubin, which has been established as a rapid means to assess sensitized production of singlet oxygen and/or reactive oxygen species [2a, 19]. As can be derived from Fig. 7, hypericin (**1**), and to a somewhat lesser extent, the didemethyl-di(acetylamino)hypericin derivative **4** are quite efficient singlet oxygen or reactive oxygen species sensitizers, which is *inter alia* very important for the potential application of **4** in photodynamic therapy. The hypericinoidic diazepine derivative **5** on the other hand did not show any photosensitizing ability, as shown in Fig. 7. The rather small quantum yields of fluorescence of **4** ($\Phi_{F,\,\text{acetone}} = 0.05$, $\Phi_{F,\,DMSO} = 0.04$), which amounts to only about one fifth of that of hypericin (**1**) [1, 20], correlates with the observed sensitizing properties. Although the fluorescence quantum yields of the corresponding diazepine derivative **5** ($\Phi_{F,\,\text{acetone}} = 0.03$, $\Phi_{F,\,DMSO} = 0.025$) are even smaller than those of **4** no photosensitizing ability could be observed. This means that in the diazepine derivative **5** there are competing pathways available, which empty efficiently the excited singlet state before intersystem crossing could occur. Either excited state

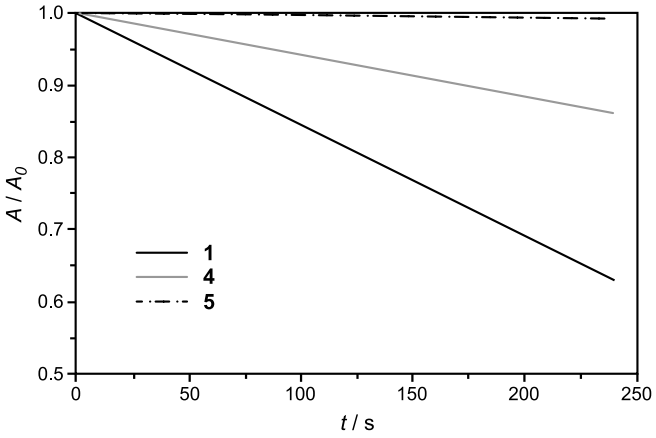


Fig. 7. Hypericin derivative sensitized photooxidation of bilirubin IXα: normalized absorption (A/A_0) *vs.* time curves of solutions of disodium bilirubinate IXα together with either sodium hypericinate ($\mathbf{1}^{(-3)}$), the sodium salt of di(acetylamino)didemethylhypericin ($\mathbf{4}^{(-3)}$), and the sodium salt of the hypericinoidic diazepine ($\mathbf{5}^{(-3)}$) in aerated 80% aqueous ethanol upon irradiation at $\lambda > 570\,\text{nm}$

proton transfer or intramolecular excitation charge transfer might be reasons that excitation transfer to the triplet state and eventually to molecular oxygen cannot occur.

Conclusions

An efficient route to amino functionalized hypericin derivatives was opened. This was realized in excellent overall yields following a multi-step synthesis strategy starting from the recently synthesized emodin derived tri-*O*-methyl emodic amine (**2**). In particular, the synthesis of the di(acetylamino)hypericin derivative **4** (47% overall yield based on emodin, eight steps) and its subsequent conversion to the corresponding hypericinoidic diazepine derivative **5** (39% overall yield based on emodin), where we realized a seven-membered ring closure strategy as a final ninth step, should be highlighted. The diaminohypericin derivative **3** could only be obtained in an amount sufficient for a limited characterization, which was due to reluctant amide bond cleavage and/or selectivity problems. However, compounds **3**–**6** and **9**–**11** could be synthesized for the first time and were fully characterized on the basis of their melting points, IR, UV/Vis, mass, and ^{1}H NMR spectra. In the case of compounds **4**, **6**, and **9**–**11** full structural assignments were achieved by ^{13}C NMR spectra and advanced 2D NMR measurements including HSQC, HMBC, and N*O*ESY experiments. The di(acetylamino)hypericin derivative **4** shows in contrast to the hypericinoidic diazepine derivative **5** improved properties concerning solubility and the bathochromically shifted long-wavelength absorption band. Simultaneously the ability of singlet oxygen and/or reactive oxygen species generation was maintained in **4** whereas **5** was found to be inefficient in this respect. This makes **4**, in particular, to an extraordinarily interesting second-generation hypericin derivative with respect to its potential application in PDT.

Experimental

All solvents were of *p.a.* quality and dried by conventional means if necessary. Reagents were supplied by commercial sources and were used without further purification. *DMF* was freshly distilled prior to use. Melting points were measured on a *Kofler* melting point microscope (Reichert, Vienna). ^{1}H NMR spectra were recorded on a Bruker Avance DRX 500 MHz spectrometer using a TXI cryoprobe with *z*-gradient coil. ^{13}C NMR spectra and 2D NMR experiments were performed on the 500 MHz spectrometer using standard pulse sequences as provided by the manufacturer. Typical 90° hard pulse durations were 8.2 μs (^{1}H) and 16.6 μs (^{13}C), 90° pulses in decoupling experiments were set to 67 μs. HSQC and HMBC experiments were optimized for coupling constants of 145 Hz for single quantum correlations and 10 Hz for multi-bond correlations. N*O*ESY mixing time was set to 400 ms. IR, UV/Vis, fluorescence, and mass spectra were recorded using the Bruker Tensor 27, Varian Cary 100 Bio UV/Vis, Hitachi 4010F, Finnigan LCQ Deca XP plus, and Fisons MD 800 instruments. Microwave assisted syntheses were run on a MLS-ETHOS 1600 microwave unit with Terminal 320 from MLS-Milestone. Hypericin sensitized photooxidation of bilirubinate IXα was executed according to Ref. [19]. It is noteworthy that due to the poor solubility of **5** in 80% aqueous *Et*OH a solution mediator (5% *DMSO*) was necessary. For the comparison of the photosensitizing properties of **1** to **4** and **5** the reference experiment with **1** was therefore executed both with and without the addition of 5% *DMSO* as solution mediator. For **1** as photosensitizer the addition of 5% *DMSO* makes no difference in the sensitized photooxidation of bilirubinate IXα. Spectrophotometric titrations of **4** were carried out in 80% aqueous *Et*OH using H_2SO_4 and tetrabutylammonium hydroxide (*TBAH*) as acid and base [16]. Because of the poor solubility of **5** in 80% aqueous *Et*OH the spectrophotometric titration of **5** was carried out in 80% aqueous *DMSO* using H_2SO_4 and *TBAH* as acid and base [16]. Semiempirical calculations were performed at the SGI Origin 3800 of the ZID at the Johannes Kepler University of Linz with Mopac/AM1 using inputs from PC-Model/MMFF94 [18]. Determination of quantum yields was performed according to Ref. [20]. The starting material tri-*O*-methyl emodic amine (**2**) was prepared according to Ref. [5]. Column as well as thin layer chromatography were carried out on silica gel 0.060–0.200 mm (pore diameter 6 nm). All novel compounds were judged to be pure (>97%) by means of their ^{1}H NMR spectra and chromatography.

10,11-Diamino-1,3,4,6,8,13-hexahydroxyphenanthro[1,10,9,8-opqra]perylene-7,14-dione (**3**, $C_{28}H_{14}N_2O_8$)

A solution of 3.33 mg (0.00564 mmol) **4** in 6 cm^3 2 *M* KOH (*Me*OH:H_2O = 4:1) under Ar was refluxed (80°C) for 2.5 h. The mixture was poured onto ice/H_2O, extracted with ethylacetate, the organic layer was washed with H_2O, dried (Na_2SO_4), filtered, and evaporated to dryness to yield 2.40 mg (84%) of a crude mixture containing the starting material **4**, the fully deprotected derivative **3**, and its partially deprotected derivatives. Compound **3** could only be isolated from this mixture as a dark green solid in unsatisfying amounts, which after several purification steps using column as well as thin layer chromatography on silica gel ($CHCl_3$:*Me*OH = 3:2) were only sufficient for performing a limited characterization. Yield (**3**) < 10%. Mp > 350°C; TLC: R_f = 0.58 ($CHCl_3$:CH_3OH = 1:1); ^{1}H NMR (500 MHz, *DMSO*-d_6, 30°C): δ = 17.89 (s, *bay*-OH), 14.83 (s, 1-OH and 6-OH), 14.50 (s, 8-OH and 13-OH), 6.97 (s_{br}, ar-NH_2) 6.78 (s, ar-H9 and ar-H12), 6.58 (s, ar-H2 and ar-H5) ppm; ESI-MS (CH_3OH:*DMSO* + 1% NH_3, $\gamma \sim 0.1$ mg · cm^{-3}, negative ion mode): m/z = 505 ([M − H]$^-$); IR (KBr): $\bar{\nu}$ = 3422, 2947, 2879, 1717, 1597, 1453, 1415, 1388, 1279, 1112, 1082, 1007, 848 cm^{-1}; UV-Vis (*DMSO*): λ_{max} = 617 (64), 345 (100) nm (rel. int.).

10,11-Bis(acetylamino)-1,3,4,6,8,13-hexahydroxyphenanthro[1,10,9,8-opqra]perylene-7,14-dione (**4**, $C_{32}H_{18}N_2O_{10}$)

A solution of 18.9 mg (0.03190 mmol) **11** in 500 cm^3 acetone was irradiated for 90 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 17.6 mg (93%) crude product were chromatographed on silica gel using $CHCl_3$:*Me*OH = 3:2 to yield 14.2 mg (75%) **4** as a dark green solid. It is noteworthy that in the case of large-scale purification of **4** a significant amount of the purified hypericin derivative can not be eluted from the column even by increasing the polarity to pure *Me*OH. In this case the silica gel should be removed from the column, suspended in acetone, treated on the ultrasonic bath to dissolve **4** and separate it from the silica gel by centrifugation. To get rid of possibly dissolved silica gel the acetone solution should be flush filtered over Celite® 545 and evaporated to dryness. Mp > 350°C; TLC: $R_f = 0.49$ ($CHCl_3$:CH_3OH = 3:2); ^{1}H NMR (500 MHz, *DMSO*-d_6, 30°C): $\delta = 18.40$ (s, *bay*-OH), 14.72 (s, 1-OH and 6-OH), 14.27 (s, 8-OH and 13-OH), 9.96 (s, 10-N*H*COCH$_3$ and 11-N*H*COCH$_3$), 8.13 (s, ar-H9 and ar-H12), 6.63 (s, ar-H2 and ar-H5), 2.07 (s, 10-NHCOC*H*$_3$ and 11-NHCOC*H*$_3$) ppm; ^{1}H NMR (500 MHz, *DMSO*-d_6, 40°C): $\delta = 18.40$ (s, 1H, *bay*-OH), 14.71 (s, 2H, 1-OH and 6-OH), 14.26 (s, 2H, 8-OH and 13-OH), 9.92 (s, 2H, 10-N*H*COCH$_3$ and 11-N*H*COCH$_3$), 8.13 (s, 2H, ar-H9 and ar-H12), 6.63 (s, 2H, ar-H2 and ar-H5), 2.07 (s, 6H, 10-NHCOC*H*$_3$ and 11-NHCOC*H*$_3$) ppm; ^{13}C NMR (125 MHz, *DMSO*-d_6, 30°C): $\delta = 182.8$ (C7 and C14), 174.0 (C3 and C4), 168.9 (10-NH*C*OCH$_3$ and 11-NH*C*OCH$_3$), 167.8 (C1 and C6), 163.1 (C8 and C13), 141.3 (C10 and C11), 126.8 (C3a′/C3b′ or C3a″/C3b″ or C7a′/C13a′), 126.3 (C3a″/C3b″ or C7a′/C13a′ or C3a′/C3b′), 121.2 (C7a′/C13a′ or C3a′/C3b′ or C3a″/C3b″), 119.2 (C6a and C14a), 112.7 (C7a and C13a), 111.3 (C9 and C12), 106.8 (C10a and C10b), 105.7 (C2 and C5), 101.9 (C3a and C3b), 23.92 (10-NHCO*C*H$_3$ and 11-NHCO*C*H$_3$) ppm; HSQC (*DMSO*-d_6, 30°C): ar-H2/H5 ↔ C2/C5, ar-H9/H12 ↔ C9/C12, 10-NHCOC*H*$_3$/12-NHCOC*H*$_3$ ↔ 10-NHCO*C*H$_3$/12-NHCO*C*H$_3$; ESI-MS (CH_3OH + 1% NH_3, $\gamma \sim 0.1\ mg \cdot cm^{-3}$, negative ion mode): $m/z = 589$ ($[M-H]^-$); IR (KBr): $\bar{\nu} = 3447$, 2956, 2925, 2853, 1682, 1603, 1560, 1535, 1464, 1408, 1375, 1337, 1256, 1115, 1092, 956, 846, 823, 795, 722, 659 cm^{-1}; UV-Vis (acetone, $c = 1.097 \cdot 10^{-5}\ mol \cdot dm^{-3}$): $\lambda_{max}(\varepsilon) = 340$ (7648), 395 (638), 485 (1012), 523 (1276), 563 (7037), 609 (16755) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$); UV-Vis (acetonitrile, $c = 1.097 \cdot 10^{-5}\ mol \cdot dm^{-3}$): $\lambda_{max}(\varepsilon) = 220$ (16399), 238 (17438), 255 (15387), 294 (12680), 335 (7411), 399 (410), 484 (839), 522 (1076), 561 (6253), 606 (13847) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$); UV-Vis (*DMF*, $c = 1.097 \cdot 10^{-5}\ mol \cdot dm^{-3}$): $\lambda_{max}(\varepsilon) = 397$ (2288), 486 (1057), 528 (985), 566 (6354), 612 (15789) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$); UV-Vis (*DMSO*, $c = 1.097 \cdot 10^{-5}\ mol \cdot dm^{-3}$): $\lambda_{max}(\varepsilon) = 295$ (14926), 336 (8627), 404 (368), 443 (295), 486 (487), 529 (678), 567 (6494), 614 (15883) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$); UV-Vis (ethanol, $c = 1.097 \cdot 10^{-5}\ mol \cdot dm^{-3}$): $\lambda_{max}(\varepsilon) = 274$ (28283), 332 (15189), 493 (2543), 527 (2120), 567 (8459), 612 (17515) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$); UV-Vis (ethanol:$H_2O$ = 4:1, $c = 1.030 \cdot 10^{-5}\ mol \cdot dm^{-3}$): $\lambda_{max}(\varepsilon) = 219$ (29973), 236 (31293), 251 (28614), 257 (28050), 292 (17517), 331 (12458), 398 (1351), 441 (1468), 477 (1303), 518 (1113), 557 (7371), 601 (15235) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$); UV-Vis (ethylacetate, $c = 1.097 \cdot 10^{-5}\ mol \cdot dm^{-3}$): $\lambda_{max}(\varepsilon) = 260$ (11877), 293 (12734), 336 (7155), 397 (400), 439 (291), 483 (911), 523 (1193), 560 (6161), 604 (13180) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$); UV-Vis (methanol, $c = 1.097 \cdot 10^{-5}\ mol \cdot dm^{-3}$): $\lambda_{max}(\varepsilon) = 237$ (17557), 256 (15469), 293 (12844), 334 (7657), 396 (292), 444 (647), 479 (1094), 519 (1222), 557 (6828), 602 (15424) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$); UV-Vis (pyridine, $c = 1.097 \cdot 10^{-5}\ mol \cdot dm^{-3}$): $\lambda_{max}(\varepsilon) = 350$ (7062), 405 (271), 447 (243), 489 (863), 530 (954), 569 (6734), 616 (16287) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$); UV-Vis (*THF*, $c = 1.097 \cdot 10^{-5}\ mol \cdot dm^{-3}$): $\lambda_{max}(\varepsilon) = 221$ (22005), 238 (21203), 296 (11331), 343 (6746), 398 (547), 490 (1276), 527 (1513), 566 (7119), 613 (16518) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$); spectrophotometric titration (ethanol:H_2O = 4:1, $c = 1.600 \cdot 10^{-5}\ mol \cdot dm^{-3}$, λ = 550–650 nm): $\lambda_{max}(\varepsilon)$ ($pH = 1.0$) = 569 (5881) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$), $\lambda_{max}(\varepsilon)$ ($pH = 3.0$) = 559 (7536), 571 (7514), 601 (9963) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$), $\lambda_{max}(\varepsilon)$ ($pH = 8.0$) = 557 (8417), 601 (16519) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$), $\lambda_{max}(\varepsilon)$ $pH = 12.5$) = 601 (6693) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$), $\lambda_{max}(\varepsilon)$ ($pH = 14.0$) = 642 (6330) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$); fluorescence (acetone, $c = 8.779 \cdot 10^{-7}\ mol \cdot dm^{-3}$, $\lambda_{ex} = 550$ nm): $\lambda_{em} = 616$ (100), 666 (23) nm (rel. int.), $\Phi_F \approx 0.05$; fluorescence (acetonitrile, $c = 8.779 \cdot 10^{-7}\ mol \cdot dm^{-3}$, $\lambda_{ex} = 550$ nm): $\lambda_{em} = 614$ (100), 664 (26) nm (rel. int.); fluorescence (*DMF*, $c = 8.779 \cdot 10^{-7}\ mol \cdot dm^{-3}$, λ_{ex} = 550 nm): $\lambda_{em} = 619$ (100), 671 (23) nm (rel. int.); fluorescence (*DMSO*, $c = 8.779 \cdot 10^{-7}\ mol \cdot dm^{-3}$, $\lambda_{ex} = 550$ nm): $\lambda_{em} = 621$ (100), 672 (23) nm (rel. int.), $\Phi_F \approx 0.04$; fluorescence (ethanol,

$c = 8.779 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): $\lambda_{em} = 609$ (100), 658 (26) nm (rel. int.); fluorescence (ethanol:H_2O = 4:1, $c = 8.779 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): $\lambda_{em} = 609$ (100), 658 (27) nm (rel. int.); fluorescence (ethylacetate, $c = 8.779 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): $\lambda_{em} = 617$ (100), 668 (23) nm (rel. int.); fluorescence (methanol, $c = 8.779 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): $\lambda_{em} = 607$ (100), 656 (27) nm (rel. int.); fluorescence (pyridine, $c = 8.779 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): $\lambda_{em} = 623$ (100), 675 (21) nm (rel. int.); fluorescence (*THF*, $c = 8.779 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): $\lambda_{em} = 619$ (100), 671 (21) nm (rel. int.).

1,7,9,11,12,14-Hexahydroxy-4-methyl-3H-phenanthro[1′,10′,9′,8′:5,6,7,8] perylo[1,12-def][1,3]diazepine-8,15-dione (**5**, $C_{30}H_{14}N_2O_8$)

Method A: A mixture of 10.7 mg (0.01812 mmol) **4** and 3.5 g (30.29 mmol) pyridinium chloride was irradiated in the microwave unit for 2 min at 400 W under Ar. Hereby the mixture was molten within 30 s and it was held at gentle reflux (223°C) for 90 s. After cooling the resulting solid was taken up in distilled H_2O and centrifuged. Additional washing of the black solid with distilled H_2O for three times and drying under vacuum over P_2O_5 ended up in 7.9 mg (82%) **5** as a dark green-blue solid.

Method B: A mixture of 3.6 mg (0.0061 mmol) **4** and 1.5 g (13.0 mmol) pyridinium chloride was molten at 150°C and heated at that temperature for 6 h under Ar. After cooling the work-up was performed according to method A to afford 2.6 mg (81%) **5**. Mp > 350°C; ^{1}H NMR (500 MHz, *DMSO*-d_6, 30°C): $\delta = 17.83$ (s, *bay*-OH), 14.73 (s_{br}, 9-OH and 14-OH), 14.66 (s, 1-OH and 7-OH), 9.30 (s_{br}, –NH–), 6.95 (s, ar-H2 and ar-H6), 6.65 (s, ar-H10 and ar-H13), 2.58 (s, 4-CH_3) ppm; the ^{13}C spectrum of **5** could not be obtained due to its very poor solubility; ESI-MS (CH_3OH:*DMSO* = 6:1 + 1% NH_3, $\gamma \sim 0.1$ mg · cm^{-3}, negative ion mode): $m/z = 529$ ($[M-H]^-$), 514 ($[M-CH_4)^-$], 487 ($[M-C_2H_4N]^-$), 486 ($[M-C_2H_5N)]^-$); IR (KBr): $\bar{\nu} = 3442$, 3374, 3057, 2926, 1694, 1621, 1574, 1504, 1459, 1425, 1392, 1315, 1249, 1197, 1118, 1096, 940, 845, 806, 722, 678, 617 cm^{-1}; UV-Vis (acetone, $c = 8.814 \cdot 10^{-6}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 337$ (11209), 482 (919), 518 (1588), 558 (7409), 602 (17121) nm (dm^3 · mol^{-1} · cm^{-1}); UV-Vis (acetonitrile, $c = 1.017 \cdot 10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 242$ (34759), 271 (35428), 335 (12832), 478 (875), 517 (1091), 555 (6647), 599 (16106) nm (dm^3 · mol^{-1} · cm^{-1}); UV-Vis (*DMF*, $c = 1.017 \cdot 10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 293$ (45231), 340 (35025), 480 (2871), 522 (2920), 561 (16195), 606 (39390), 680 (1544) nm (dm^3 · mol^{-1} · cm^{-1}); UV-Vis (*DMSO*, $c = 1.017 \cdot 10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 288$ (53687), 338 (30865), 410 (4090), 478 (2144), 523 (2468), 563 (14179), 608 (33943), 685 (974) nm (dm^3 · mol^{-1} · cm^{-1}); UV-Vis (ethanol, $c = 1.017 \cdot 10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 238$ (38446), 273 (34267), 331 (12596), 514 (1298), 551 (6686), 595 (15900) nm (dm^3 · mol^{-1} · cm^{-1}); UV-Vis (pyridine, $c = 1.017 \cdot 10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 380$ (24287), 483 (1357), 524 (1170), 563 (7719), 609 (19803), 685 (924) nm (dm^3 · mol^{-1} · cm^{-1}); UV-Vis (*THF*, $c = 1.017 \cdot 10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 336$ (10364), 486 (1062), 521 (1760), 560 (8496), 606 (19646), 675 (4061) nm (dm^3 · mol^{-1} · cm^{-1}); spectrophotometric titration (*DMSO*:H_2O = 4:1, $c = 1.500 \cdot 10^{-5}$ mol · dm^{-3}, $\lambda = 550$–650 nm): $\lambda_{max}(\varepsilon)$ ($pH = 1.0$) = 576 (3847) nm (dm^3 · mol^{-1} · cm^{-1}), $\lambda_{max}(\varepsilon)$ ($pH = 3.0$) = 577 (4313), 613 (3608) nm (dm^3 · mol^{-1} · cm^{-1}), $\lambda_{max}(\varepsilon)$ ($pH = 8.0$) = 560 (6648), 604 (14350) nm (dm^3 · mol^{-1} · cm^{-1}), $\lambda_{max}(\varepsilon)$ $pH = 12.5$) = 561 (7062), 604 (16841) nm (dm^3 · mol^{-1} · cm^{-1}), $\lambda_{max}(\varepsilon)$ ($pH = 14.0$) = 648 (13407) nm (dm^3 · mol^{-1} · cm^{-1}); fluorescence (acetone, $c = 7.050 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): $\lambda_{em} = 609$ (100), 656 (39) nm (rel. int.), $\Phi_F \approx 0.030$; fluorescence (acetonitrile, $c = 8.136 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} =$ 550 nm): $\lambda_{em} = 608$ (100), 654 (41) nm (rel. int.); fluorescence (*DMF*, $c = 8.136 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): $\lambda_{em} = 614$ (100), 657 (52) nm (rel. int.); fluorescence (*DMSO*, $c = 8.136 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): $\lambda_{em} = 616$ (100), 655 (49) nm (rel. int.), $\Phi_F \approx 0.025$; fluorescence (ethanol, $c = 8.136 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): $\lambda_{em} = 603$ (100), 643 (44) nm (rel. int.); fluorescence (pyridine, $c = 8.136 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): $\lambda_{em} = 614$ (100), 657 (42) nm (rel. int.); fluorescence (*THF*, $c = 8.136 \cdot 10^{-7}$ mol · dm^{-3}, $\lambda_{ex} = 550$ nm): $\lambda_{em} = 611$ (100), 658 (35) nm (rel. int.).

1,3,8-Trihydroxy-6-amino-10H-anthracen-9-one (**6**, $C_{14}H_{11}NO_4$)
To a solution of 40.0 mg (0.128 mmol) **2** in 8.0 cm^3 glacial acetic acid at 85°C under Ar, 3.66 cm^3 HBr (47% aq) and 230.5 mg (1.021 mmol) $SnCl_2 \cdot 2H_2O$ were added. The resulting mixture was heated to 105°C and kept at that temperature for 30 min, cooled, poured onto ice/H_2O, and neutralized with 4 *N* NaOH. The precipitate was centrifuged off, washed with distilled H_2O, and dried over P_2O_5 under vacuum. The crude solid was suspended in pyridine, filtered off, and the filtrate was evaporated to dryness to yield 32.2 mg (98%) **6** as a brown solid. Mp > 208°C (decomp); TLC: $R_f = 0.84$ ($CHCl_3$:CH_3OH = 2:1), $R_f = 0.46$ ($CHCl_3$:CH_3OH = 10:1), $R_f = 0.16$ ($CHCl_3$:$CH_3COOC_2H_5$ = 3:1); ^{1}H NMR (500 MHz, *DMSO*-d_6, 30°C): $\delta = 12.71$ (s, 1-OH), 12.51 (s, 8-OH), 10.44 (s, 3-OH), 6.42 (s, 6-NH_2), 6.33 (d, $J = 2.2$ Hz, ar-H4), 6.15 (d, $J = 2.2$ Hz, ar-H2), 6.12 (d, $J = 1.9$ Hz, ar-H5), 5.91 (d, $J = 1.9$ Hz, ar-H7), 4.14 (s, –CH_2–) ppm; N*O*ESY (*DMSO*-d_6, 30°C): 1-OH ↔ ar-H2, 3-OH ↔ ar-H2 and ar-H4, 8-OH ↔ ar-H7, 6-NH_2 ↔ ar-H5 and ar-H7, 10-CH_2 ↔ ar-H4 and ar-H5; ^{13}C NMR (125 MHz, *DMSO*-d_6, 30°C): $\delta = 188.2$ (C9), 164.2 (C8), 163.8 (C1), 163.5 (C3), 156.1 (C6), 143.7 (C4a or C10a), 143.6 (C10a or C4a), 108.0 (C9a), 106.8 (C4), 105.1 (C8a), 104.9 (C5), 100.7 (C2), 97.19 (C7), 32.28 (C10) ppm; HSQC (*DMSO*-d_6, 30°C): ar-H2 ↔ C2, ar-H4 ↔ C4, ar-H5 ↔ C5, ar-H7 ↔ C7, 10-CH_2 ↔ 10-CH_2; HMBC (*DMSO*-d_6, 30°C): C1 → 1-OH and ar-H2, C2 → ar-H4, 1-OH, and 3-OH, C3 → 3-OH, ar-H2, ar-H4, and 10-CH_2, C4 → ar-H2, 3-OH, and 10-CH_2, C5 → ar-H7 and 10-CH_2, C6 → ar-H7, 8-OH, and 10-CH_2, C7 → ar-H5, 6-NH_2, and 8-OH, C8 → 8-OH, and ar-H7, C10 → ar-H4, and ar-H5, C8a → 6-NH_2 and 10-CH_2, C9a → 1-OH, ar-H2, ar-H4, and 10-CH_2, C4a/C10a → ar-H4, ar-H5, and 10-CH_2; ESI-MS (CH_3OH:*DMSO* = 1:1 + 2% NH_3, $\gamma \sim 0.1$ mg · cm^{-3}, negative ion mode): $m/z = 256$ ($[M-H]^-$); IR (KBr): $\bar{\nu} = 3458$, 3368, 3234, 2923, 2852, 1619, 1604, 1476, 1389, 1330, 1262, 1219, 1174, 1155, 1059, 915, 835, 824, 619, 551 cm^{-1}; UV-Vis (CH_3OH, $c = 1.20 \cdot 10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 223$ (11583), 259 (7500), 280 (6750), 377 (8083) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$); UV-Vis ($CHCl_3$, $c = 1.20 \cdot 10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 260$ (6333), 282 (5833), 370 (5833) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$).

1,3,8-Trimethoxy-6-(acetylamino)-9,10-anthraquinone (**9**, $C_{19}H_{17}NO_6$)
To a solution of 15.0 mg (0.0485 mmol) **2** in 2 cm^3 dry 1,4-dioxan at 85°C under Ar, 6.8 mm^3 (0.0958 mmol) acetyl chloride and 7.7 mm^3 (0.0958 mmol) dry pyridine were added. The mixture was heated and refluxed for 15 min. After cooling the solution was poured onto ice/H_2O, centrifuged three times with distilled H_2O, and dried over P_2O_5 under vacuum to yield 17.0 mg (98%) **9** as a yellow solid. Mp 281–283°C; TLC: $R_f = 0.68$ ($CHCl_3$:C_2H_5OH = 5:1), $R_f = 0.09$ ($CHCl_3$:$CH_3COOC_2H_5$ = 1:1); ^{1}H NMR (500 MHz, *DMSO*-d_6, 30°C): $\delta = 10.46$ (s, 6-N*H*COCH$_3$), 7.89 (d, $J = 1.8$ Hz, ar-H5), 7.83 (d, $J = 1.8$ Hz, ar-H7), 7.18 (d, $J = 2.4$ Hz, ar-H4), 6.98 (d, $J = 2.4$ Hz, ar-H2), 3.94 (s, 3-OCH_3), 3.89 (s, 1-OCH_3), 3.85 (s, 8-OCH_3), 2.11 (s, 6-NHCOC*H*$_3$) ppm; N*O*ESY (*DMSO*-d_6, 30°C): 1-OCH_3 ↔ ar-H2, 3-OCH_3 ↔ ar-H2 and ar-H4, 8-OCH_3 ↔ ar-H7, 6-N*H*COCH$_3$ ↔ ar-H5, ar-H7, and 6-NHCOC*H*$_3$; ^{13}C NMR (125 MHz, *DMSO*-d_6, 30°C): $\delta = 183.1$ (C10), 179.1 (C9), 169.2 (6-NH*C*OCH$_3$), 163.2 (C3), 161.1 (C1), 160.0 (C8), 144.0 (C6), 135.5 (C4a), 134.7 (C10a), 118.4 (C8a), 117.5 (C9a), 108.0 (C5), 107.8 (C7), 105.1 (C2), 102.3 (C4), 56.33 (1-OCH_3), 56.00 (8-OCH_3), 55.83 (3-OCH_3), 24.18 (6-NHCO*C*H$_3$) ppm; HSQC (*DMSO*-d_6, 30°C): ar-H2 ↔ C2, ar-H4 ↔ C4, ar-H5 ↔C5, ar-H7 ↔ C7, 1-OCH_3 ↔ 1-OCH_3, 3-OCH_3 ↔ 3-OCH_3, 8-OCH_3 ↔ 8-OCH_3, 6-NHCOC*H*$_3$ ↔ 6-NHCO*C*H$_3$; HMBC (*DMSO*-d_6, 30°C): C1 → 1-OCH_3 and ar-H2, C2 → ar-H4, C3 → 3-OCH_3, ar-H2, and ar-H4, C4 → ar-H2, C5 → ar-H7 and 6-NH–, C6 → ar-H5 and ar-H7, C7 → ar-H5, C8 → 8-OCH_3 and ar-H7, C9 → ar-H2 and ar-H7, C10 → ar-H4 and ar-H5, C4a → ar-H4, C8a → ar-H5 and ar-H7, C9a → ar-H2 and ar-H4, 6-NH*C*OCH$_3$ → 6-N*H*COCH$_3$ and 6-NHCOC*H*$_3$; ESI-MS (CH_3OH:*DMSO* = 3:1 + 2% HCOOH, $\gamma \sim 0.1$ mg · cm^{-3}, positive ion mode): $m/z = 356$ ($[M+H]^+$); NCI-MS (solids probe, CH_4): $m/z = 355$ ($[M]^-$); IR (KBr): $\bar{\nu} = 3318$, 3187, 3112, 3019, 2926, 2853, 1731, 1673, 1656, 1596, 1567, 1538, 1456, 1405, 1343, 1320, 1253, 1234, 1221, 1202, 1169, 1136, 1117, 1072, 1053, 1033, 1012, 994, 946, 879, 868, 842, 716, 558 cm^{-1}; UV-Vis (CH_3OH, $c = 3.52 \cdot 10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 228$ (22071), 288 (21628), 415 (4159) nm

($dm^3 \cdot mol^{-1} \cdot cm^{-1}$); UV-Vis ($CHCl_3$, $c = 3.52 \cdot 10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 242$ (18182), 288 (27278), 415 (4767) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$).

1,3,8-Trihydroxy-6-(acetylamino)-10H-anthracen-9-one (**10**, $C_{16}H_{13}NO_5$)

A mixture of 8.0 mg (0.0311 mmol) **6**, 4.4 mm^3 (0.0622 mmol) acetyl chloride, and 5.0 mm^3 (0.0622 mmol) dry pyridine in 2 cm^3 dry 1,4-dioxan was refluxed for 1 h under Ar. After cooling the solution was poured onto ice/H_2O, centrifuged three times with distilled H_2O, and dried over P_2O_5 under vacuum to yield 8.6 mg (92%) **10** as an ochre solid. Mp > 252°C (decomp); TLC: $R_f = 0.78$ ($CHCl_3$:CH_3OH = 2:1), $R_f = 0.53$ ($CHCl_3$:CH_3OH = 10:1); ^{1}H NMR (500 MHz, *DMSO*-d_6, 30°C): $\delta = 12.40$ (s, 1-OH), 12.35 (s, 8-OH), 10.74 (s, 3-OH), 10.26 (s, 6-N*H*COCH$_3$), 7.16 (s, ar-H5 and ar-H7), 6.42 (d, $J = 2.0$ Hz, ar-H4), 6.21 (d, $J = 2.0$ Hz, ar-H2), 4.32 (s, –CH_2–), 2.09 (s, –$COCH_3$) ppm; ^{1}H NMR (500 MHz, *DMSO*-d_6, 50°C): $\delta = 12.37$ (s, 1H, 1-OH), 12.32 (s, 1H, 8-OH), 10.64 (s, 1H, 3-OH), 10.18 (s, 1H, 6-N*H*COCH$_3$), 7.17 (s, 1H, ar-H5), 7.16 (s, 1H, ar-H7), 6.42 (s, 1H, ar-H4), 6.22 (s, 1H, ar-H2), 4.31 (s, 2H, –CH_2–), 2.09 (s, 3H, –$COCH_3$) ppm; N*O*ESY (*DMSO*-d_6, 50°C): 1-OH ↔ ar-H2, 3-OH ↔ ar-H2 and ar-H4, 8-OH ↔ ar-H7, 6-N*H*COCH$_3$ ↔ ar-H5, ar-H7, and 6-NHCOC*H*$_3$, 10-CH_2 ↔ ar-H4 and ar-H5; ^{13}C NMR (125 MHz, *DMSO*-d_6, 30°C): $\delta = 190.0$ (C9), 169.3 (6-NH*C*OCH$_3$), 164.7 (C3), 164.4 (C1), 162.8 (C8), 145.7 (C6), 144.7 (C4a or C10a), 143.2 (C10a or C4a), 110.5 (C8a), 109.0 (C5), 108.2 (C9a), 107.3 (C4), 103.7 (C7), 100.9 (C2), 32.50 (C10), 24.29 (6-NHCO*C*H$_3$) ppm; ^{13}C NMR (125 MHz, *DMSO*-d_6, 50°C): $\delta = 189.9$ (C9), 169.0 (6-NH*C*OCH$_3$), 164.5 (C3), 164.3 (C1), 162.6 (C8), 145.6 (C6), 144.5 (C4a), 143.0 (C10a), 110.4 (C8a), 108.9 (C5), 108.1 (C9a), 107.1 (C4), 103.7 (C7), 100.8 (C2), 32.41 (C10), 24.08 (6-NHCO*C*H$_3$) ppm; HSQC (*DMSO*-d_6, 50°C): ar-H2 ↔ C2, ar-H4 ↔ C4, ar-H5 ↔ C5, ar-H7 ↔ C7, 10-CH_2 ↔ 10-CH_2, 6-NHCOC*H*$_3$ ↔ 6-NHCO*C*H$_3$; HMBC (*DMSO*-d_6, 50°C): C1 → 1-OH and ar-H2, C2 → ar-H4, 1-OH, and 3-OH, C3 → 3-OH, ar-H2, ar-H4, and 10-CH_2, C4 → ar-H2, 3-OH, and 10-CH_2, C5 → ar-H7, 6-N*H*COCH$_3$, and 10-CH_2, C6 → ar-H5, ar-H7, 6-N*H*COCH$_3$, 8-OH, and 10-CH_2, C7 → ar-H5, 6-N*H*COCH$_3$, and 8-OH, C8 → 8-OH and ar-H7, C9 → 10-CH_2, C10 → ar-H4 and ar-H5, C8a → ar-H7, 8-OH, and 10-CH_2, C9a → 1-OH, ar-H2, ar-H4, and 10-CH_2, C4a → ar-H4, ar-H2, and 10-CH_2, C10a → ar-H4, ar-H5, and 10-CH_2, 6-NH*C*OCH$_3$ → 6-N*H*COCH$_3$ and 6-NHCOC*H*$_3$; APCI-MS (CH_2Cl_2:CH_3OH = 1:1, $\gamma \sim 0.1$ mg · cm^{-3}, positive ion mode): $m/z = 300$ ($[M+H]^+$); IR (KBr): $\bar{\nu} = 3322$, 3117, 2969, 2922, 2855, 1679, 1611, 1538, 1481, 1419, 1372, 1341, 1272, 1207, 1183, 1155, 1109, 1064, 998, 953, 924, 909, 863, 847, 800, 758, 673, 586 cm^{-1}; UV-Vis (CH_3OH, $c = 1.28 \cdot 10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) = 210$ (31552), 220 (31230), 251 (8863), 284 (12971), 363 (22691) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$); UV-Vis ($CH_3COOCH_2CH_3$, $c = 1.28 \cdot 10^{-5}$ mol · dm^{-3}): $\lambda_{max}(\varepsilon) =$ 284 (10963), 363 (20349) nm ($dm^3 \cdot mol^{-1} \cdot cm^{-1}$).

10,13-Bis(acetylamino)-1,3,4,6,8,15-hexahydroxydibenzo[ao]perylene-7,16-dione (**11**, $C_{32}H_{20}N_2O_{10}$)

A mixture of 26.7 mg (0.08921 mmol) **10**, 1.2 mg (0.00446 mmol) $FeSO_4 \cdot 7H_2O$, and 46.7 mg (0.49068 mmol) pyridine-*N*-oxide in 1.05 cm^3 dry pyridine and 95 mm^3 dry piperidine was stirred under Ar with protection from light at 112°C for 1 h. After cooling to room temperature the reaction mixture was poured into 4 cm^3 2 *M* HCl and stirred for 30 min at room temperature in the dark. After centrifugation the residue was washed three times each with 3% HCl, distilled H_2O, and dried under vacuum over P_2O_5 to yield 23.2 mg (88%) **11** as a black solid. Mp > 350°C; ^{1}H NMR (500 MHz, *DMSO*-d_6, 40°C): $\delta = 14.30$ (s, 1-OH and 6-OH), 12.93 (s, 8-OH and 15-OH), 9.94 (s, 10-N*H*COCH$_3$ and 13-N*H*COCH$_3$), 7.49 (s, ar-H11 and ar-H12), 7.46 (s, ar-H9 and ar-H14), 6.31 (s, ar-H2 and ar-H5), 1.95 (s, 10-NHCOC*H*$_3$ and 13-NHCOC*H*$_3$) ppm – the 3-OH and 4-OH signals could not be detected obviously due to line broadening and exchange phenomena; ^{13}C NMR (125 MHz, *DMSO*-d_6, 40°C): $\delta = 183.7$ (C7 and C16), 173.3 (C3 and C4), 168.8 (10-NH*C*OCH$_3$ and 13-NH*C*OCH$_3$), 168.0 (C1 and C6), 161.0 (C8 and C15), 142.8 (C10 and C13), 136.8 (C3a′ and C3b′ or C11b and C11c), 129.8 (C11b and C11c or C3a′ and C3b′), 127.8 (C11a and C11d), 119.4 (C6a and C16a), 115.1 (C11 and C12), 111.0 (C7a and C15a), 105.5 (C9 and C14), 104.1 (C2 and C5), 99.26 (C3a and C3b), 24.07

(10-NHCO*C*H$_3$ and 13-NHCO*C*H$_3$) ppm; HSQC (*DMSO*-d$_6$, 40°C): ar-H2/H4 ↔ C2/C4, ar-H9/H14 ↔ C9/C14, ar-H11/H12 ↔ C11/C12, 10/13-NHCOC*H*$_3$ ↔ 10/13-NHCO*C*H$_3$; HMBC (*DMSO*-d$_6$, 40°C): C1/C6 → ar-H2/ar-H5, C3/C4 → ar-H2/ar-H5, C3a/C3b → ar-H2/ar-H5, C6a/C16a → ar-H2/ar-H5, C7a/C15a → ar-H9/ar-H14, C8/C15 → ar-H9/ar-H14, C9/C14 → ar-H11/ar-H12, C10/C13 → ar-H9/ar-H11/ar-H12/ar-H14, C11/C12 → ar-H9/ar-H14 and 10/13-N*H*COCH$_3$, C11a/C11d → ar-H11/ar-H12, 10/13-NH*C*OCH$_3$ → 10/13-N*H*COCH$_3$ and 10/13-NHCOC*H*$_3$; ESI-MS ($CH_3OH + 1\%$ NH_3, $\gamma \sim 0.1\,mg \cdot cm^{-3}$, negative ion mode): $m/z = 591$ ($[M-H]^-$); IR (KBr): $\bar{\nu} = 3454$, 3068, 2921, 2850, 1681, 1605, 1530, 1485, 1393, 1372, 1273, 1211, 1105, 1034, 996, 847, 752, 678 cm^{-1}; UV-Vis (acetone): $\lambda_{max} = 382$ (100), 557 (75), 592 (66) nm (rel. int.).

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