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New flavonoid–porphyrin conjugates via Buchwald–Hartwig amination: synthesis and photophysical studies

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ABSTRACT

New flavonoid–porphyrin conjugates were synthesized using the cross-coupling Buchwald–Hartwig amination for the coupling of flavonoid and porphyrin moieties. A unique di-substituted flavone–porphyrin conjugate was also synthesized under similar reaction conditions for the first time. All the conjugates were fully characterized by NMR spectroscopy. The photophysical properties namely fluorescence and singlet oxygen production were evaluated considering their use for photodynamic therapy applications.

Introduction

Porphyrin derivatives besides their important role in biological systems are finding applications in different areas, namely in medicine.[1] In the last two decades, the photodynamic therapy (PDT) using porphyrin derivatives as photosensitizing biological chromophores is considered as a non-invasive and efficient cancer therapy.[2] The medical application is the most popular example, however, photodynamic inactivation of pathogenic microorganisms is also gaining attention.[3] Owing to their important applications various efforts to obtain new derivatives with improved biological properties have been undertaken. In this perspective, the synthesis of several porphyrin conjugates with other molecules has been reported emphasizing that the final product has improved properties with dual functions. For instance, glycoporphyrins, not only offer better solubility in an aqueous environment but also improved targeting.[4] Other examples can be mentioned, like the porphyrin–ferrocene conjugates that reveal electron-transfer from the ferrocene to porphyrin by changing their fluorescence emission[5] and the chalcone-porphyrin conjugates that appear to be potential agents for cancer diagnosis wherein the chalcone moiety induces a protective effect.[6] In addition there are several reports pointing out that flavonoids can be used in medical formulations[7] and among them flavones are the most interesting ones due to their interesting biological activities.[8] Thus the conjugation of porphyrins to other important entities seems to be a good strategy toward the discovery of new drugs. Recently we reported the synthesis of novel flavone-dihydroporphyrin conjugates,[9] therefore we decided to focus our interest in the synthesis of new flavonoid–porphyrin conjugates. Considering that the Buchwald–Hartwig palladium-catalyzed amination is a powerful approach to conjugate porphyrin with other molecules via carbon–nitrogen bonds,[10] herein we describe the first application of this methodology to obtain new molecules incorporating porphyrin and flavonoid moieties.

Results and discussion

The flavonoids used as starting compounds were synthesized by previously reported procedures, wherein the aldol condensation of 2′-hydroxyacetophenone with 4-bromobenzaldehyde afforded the 4-bromo-2′-hydroxychalcone **1**, which is transformed into 4-bromoflavone **2** by cyclodehydrogenation (Fig. 1).[11] The amino porphyrins (2-amino-5,10,15,20-tetraphenylporphyrinato)-nickel(II) **3** and [5-(4-aminophenyl)-10,15,20-triphenylporphyrinato]zinc(II) **4**, were also prepared according to known procedures[12] in which the 5,10,15,20-tetraphenylporphyrin (**TPP**)[13] is used as the starting porphyrin.

The synthetic strategy to prepare the new flavonoid–porphyrin conjugates was based on the Buchwald–Hartwig palladium-cata-

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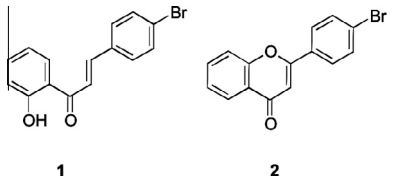
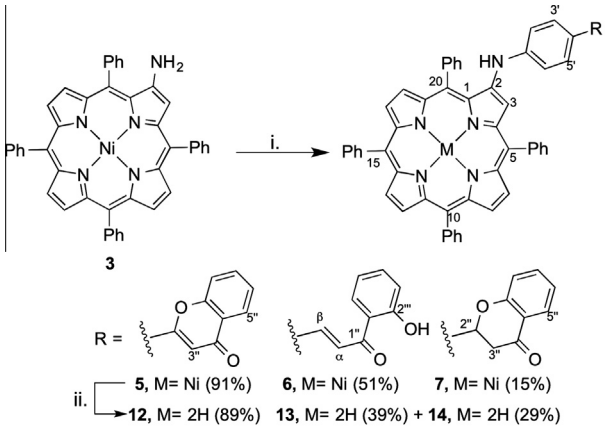


Figure 1. Flavonoid derivatives used in this study.



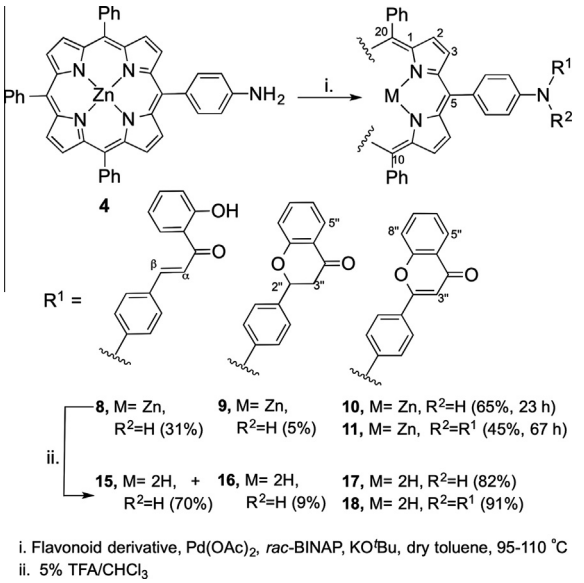
i. Flavonoid derivative, $Pd(OAc)_2$, *rac*-BINAP, KO^{t}Bu, dry toluene, 95-110 °C
ii. 10% $H_2SO_4/CHCl_3$

Scheme 1. Synthetic strategy to prepare flavonoid–porphyrin conjugates at β-position.

lyzed amination, using the established conditions for the coupling of porphyrins with bromobenzene derivatives.[14]

Accordingly the reactions of porphyrin **3** with 4-bromo-2′-hydroxychalcone **1** and 4-bromoflavone **2** were carried out in dry toluene, in the presence of the catalytic system $Pd(OAc)_2$, *rac*-BINAP as the phosphine ligand, and using KO^{t}Bu as the base (Scheme 1).[15] The reactions were monitored by TLC and ended after the consumption of the starting porphyrin. The reaction with 4-bromoflavone **2** gave the desired conjugate **5** in an overall yield of 91%. The reaction with 4-bromo-2′-hydroxychalcone **1** afforded after a careful chromatographic separation the desired conjugate **6** in 51% and a secondary product in 15% yield. The mass spectrum of this byproduct shows a peak at *m*/*z* 907 [$M^{+\cdot}$] identical to the mass observed for conjugate **6**.[16] However, the ^{1}H NMR shows in the aliphatic region at δ 5.42 a double doublet characteristic of a flavanone H-2″ and the two double doublets characteristic of the flavanone H-3″ protons at δ 3.15 and 2.88 ppm. The coupling constants ($J_{geminal}$ = 16.9 Hz, $J_{vicinal}$ = 13.4 and 2.8 Hz) also point out the presence of a flavanone moiety linked to the porphyrin as proposed in structure **7** (Scheme 1). This byproduct formation can be due to the known equilibrium between 2′-hydroxichalcone and flavanone nucleus that can be favored under the coupling conditions. Attempts to improve the outcome of chalcone coupling were not successful.

The methodology was extended to the zinc(II) porphyrin **4** bearing the amino group as the *meso*-phenyl substituent. The inner core of the macrocycle was protected by zinc in order to avoid metallation by palladium. The reaction with chalcone **1** afforded the expected conjugate **8** in 31% yield (Scheme 2). The formation of the flavanone–porphyrin conjugate **9** (~5% yield), was also observed as in the case of β-aminoporphyrin derivative **3**, which was confirmed by its ^{1}H NMR spectra and a peak at *m*/*z* 913 [$M^{+\cdot}$] in the mass spectrum. When 4′-bromoflavone **2** was used



i. Flavonoid derivative, $Pd(OAc)_2$, *rac*-BINAP, KO^{t}Bu, dry toluene, 95-110 °C
ii. 5% $TFA/CHCl_3$

Scheme 2. Synthetic strategy to prepare flavonoid–porphyrin conjugates at *meso*-position.

as the reagent the desired conjugate **10** was obtained in 65% yield. Traces of a byproduct were also isolated from the reaction mixture. The ^{1}H NMR spectra indicated the presence of two flavone units and showed the absence of the singlet due to the amino group N*H*, furthermore a peak at 1131 [$M^{+\cdot}$] in its MS spectra confirmed that it was the di-substituted compound **11**.[17] Prolonged reaction times (67 h vs 23 h) led to an inversion of the reaction mixture composition, wherein the di-substituted derivative **11** was obtained as the main product (45%) while the desired conjugate **10** was obtained in 11% yield (Scheme 2). It was possible to prepare the di-substituted derivative **11** as the main product by the reaction of conjugate **10** with 4′-bromoflavone **2** under the same reaction conditions. The above mentioned results indicate that porphyrin **4** is slightly less reactive than porphyrin **3** but leads to an unusual double amination.[18]

The structures of the new conjugates were confirmed by 1D and 2D NMR spectroscopy and mass spectrometry.[16,17,19] The ^{1}H NMR spectra of conjugates **5** and **6** are similar and are consistent with β-substituted porphyrins, showing the singlet of proton H-3 resonance at δ 8.42 and 8.37 ppm, respectively, and the other six β-pyrrolic proton resonances at δ 8.54–8.71. Other important features are the singlets due to the amino N*H* at δ 6.57 and 6.55 ppm, respectively for conjugates **5** and **6**. Finally the characteristic signals due to the flavonoid moiety, which are: (i) the singlet due to proton H-3″ at δ 6.74 and the double doublet due to proton H-5″ at δ 8.24, both distinctive of the flavone moiety; and (ii) the distinctive signals of the chalcone moiety, singlet due to the 2″-O*H* proton at δ 12.89 and the vinylic system doublets at δ 7.49 and 7.87 ppm due to H-α and H-β, respectively showing a coupling constant of *J* 15.4 Hz, consistent with a *trans* configuration. Besides that, ^{13}C NMR spectra of conjugates **5** and **6** also exhibit signals at δ 178.3 and 193.7 ppm due to the carbonyl resonances of the flavone and chalcone moieties. The ^{1}H NMR spectra of conjugates **8** and **10**[19] which are linked at the 4-position of *meso*-phenyl group of the porphyrin are very similar. In addition to the characteristic signals of the flavone and chalcone moieties, the eight β-pyrrolic protons appear at δ 8.95–9.05 as well as a broad singlet due to N*H* proton at δ 6.47 and 6.39 ppm. The most significant feature, the AB system due to the protons of the *para*-substituted 5-phenyl group, in which the *ortho*-protons resonated at δ 8.20 and 8.18 ppm and the *meta* at δ 7.58 and 7.53 ppm was observed.

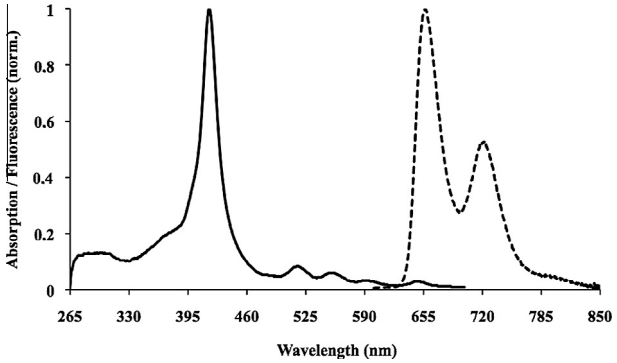


Figure 2. Representative normalized absorption (solid) and fluorescence spectra (dotted) (λ_{exc} = 550 nm, OD = 0.02) of flavonoid–porphyrin conjugate **17** in DMF.

Considering the potential application of these new flavonoid–porphyrin conjugates in medicine, namely in PDT, we carried out the decomplexation of all synthesized compounds (Scheme 1 and 2).[20] For this, conjugates **5** and **6** which are linked at the β-pyrrolic position of the Ni(II) porphyrin were treated with 10% concentrated sulfuric acid in chloroform and the desired free bases **12** and **13** were obtained in 89% and 39% yields, respectively. It is worth mentioning that during the decomplexation of conjugate **6** the formation of the corresponding flavanone–porphyrin conjugate **14** was observed in 29% yield (Scheme 1). The demetallation of zinc ion from the flavonoid–porphyrin conjugates **8** and **10**, was achieved using 5% of trifluoroacetic acid in chloroform. In this case the desired derivatives **15** and **17** were obtained in 70% and 82% yields, respectively, and the formation of the flavanone–porphyrin conjugate **16** was also observed (9%) (Scheme 2). The decomplexation of the conjugate **11** obtained led to the formation of conjugate **18** in 91% yield. The structure of these free base derivatives was confirmed by various means. In UV–Vis spectroscopy, all free base derivatives present four Q bands (Fig. 2). The ^{1}H NMR analysis showed the inner N*H* of the macrocycle confirming the removal of the metal ion from the inner core. Besides that the samples were also analyzed by mass spectrometry.[21]

To be considered for use in any photodynamic procedure the ability of the compound to generate singlet oxygen ($^1O_2$) is essential taking into account that the $^1O_2$ is the main reactive oxygen species (ROS) responsible for cell death.[22] Therefore, fluorescence quantum yields (Φ_{fl}) and singlet oxygen quantum yields (Φ_Δ) were determined. The methods used—steady state absorption and fluorescence as well as time resolved singlet oxygen luminescence detection have been described.[23] The steady state fluorescence spectra were measured in DMF solutions under normal air conditions with an OD = 0.02 at λ_{exc} = 550 and 580 nm for all conjugates with the flavonoids linked at the *meso*- or β-pyrrolic positions of the porphyrin macrocycle, respectively. The fluorescence emission spectra of the conjugates are characterized by two emission bands (Fig. 2). The fluorescence maxima of flavonoid–porphyrin appear at around 610 nm for the zinc derivatives and around 660 nm for all free bases (M = 2H).

The fluorescence quantum yields for the *meso*-substituted free-base conjugates **15**–**18** reach values between 14% and 19%, for the Zn(II) conjugates **8**–**11** between 2% and 8%. The coupling of the flavone moiety to the *meso*-position of the porphyrin macrocycle leads to an increased fluorescence (**TPP**, Φ_{fl} = 11%).[24] Moreover, the lower Φ_{fl} of the Zn(II) complexes has already been observed in other Zn derivatives.[25] The conjugates with flavonoid moieties linked at the β-position **12**–**14** show low fluorescence emission (4–5%) compared with those in the *meso* position.

The ability of all conjugates to generate singlet oxygen was also evaluated in DMF using **TPP** as a standard. **TPP** is known to be a good singlet oxygen generator (Φ_Δ = 68% in DMF).[26] From the results obtained (Fig. 3), we can see that all conjugates are capable to generate singlet oxygen, although the coupling of the flavonoid units to the porphyrin induced different effects. For instance, the presence of the chalcone or flavanone moieties, in both positions, reduced the ability of these derivatives to produce singlet oxygen resulting in the Φ_Δ values of 22–56%. Otherwise, the coupling of the flavone moiety, particularly in the *meso*-position, increases the Φ_Δ to values between 67% for the flavone–porphyrin conjugate **17** and 83% for conjugate **10**.

In summary, this work successfully accomplished for the first time the coupling of flavonoid and porphyrin moieties and a novel di-substituted flavone–porphyrin conjugate using the cross-cou-

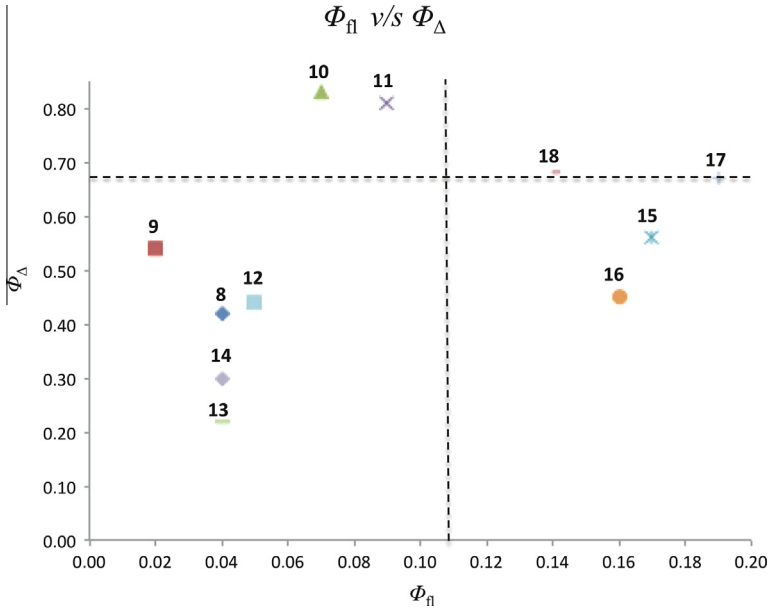


Figure 3. Plot of the singlet oxygen quantum yield (Φ_Δ) of the flavonoid–porphyrin conjugates versus their fluorescence quantum yield (Φ_{fl}) in DMF. The interception of the dotted lines represents the value of the standard (TPP).

pling Buchwald–Hartwig amination. The study of the photophysical properties reveals that most of the conjugates present fluorescence and good capacity to generate singlet oxygen, making these compounds possible candidates to be used as photosensitizers in PDT or in fluorescence diagnosis. Further design and investigation of new flavonoid–porphyrin conjugates in biological systems will be performed to validate these preliminary results.

Acknowledgments

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15. *General procedure:* To a mixture of porphyrin:flavonoid in the ratio (1:2) dissolved in dry toluene were added $Pd(OAc)_2$ (0.29 equiv), *rac*-BINAP (0.25 equiv), and KO^{t}Bu (2.13 equiv). The mixture was stirred at 95–110 °C until all porphyrin derivatives were consumed. The mixture was filtered through a Celite®-545 column, extracted with $CHCl_3$, washed, and dried over anhydrous sodium sulfate. The residue obtained was chromatographed by preparative thin layer chromatography (TLC) using $CHCl_3$ as solvent.
16. *Conjugate* ***6***: ^{1}H NMR ($CDCl_3$, 500.13 MHz): δ 12.89 (br s, 1H, O*H*), 8.6812 and 8.6809 (AB, 2H, J = 5.0 Hz, H-$\beta_{pyrrolic}$), 8.66 and 8.61 (AB, 2H, J = 5.0 Hz, H-$\beta_{pyrrolic}$), 8.64 and 8.54 (AB, 2H, J = 4.9 Hz, H-$\beta_{pyrrolic}$), 8.37 (s, 1H, H-3), 8.00–7.95 (m, 8H, 5,10,15,20-H-*o*-Ph), 7.90 (dd, 1H, J = 8.1, 1.7 Hz, H-6‴), 7.87 (d, 1H, J = 15.4 Hz, H-β), 7.81–7.78 (m, 1H, 20-H-*p*-Ph), 7.75–7.72 (m, 2H, 20-H-*m*-Ph), 7.67–7.64 (m, 9H, 5,10,15-H-*m,p*-Ph), 7.53 (d, 2H, J = 8.7 Hz, H-3′,5′), 7.49 (d, 1H, J = 15.4 Hz, H-α), 7.46–7.44 (m, 1H, H-4‴), 7.01 (dd, 1H, J = 8.4, 1.1 Hz, H-3‴), 6.91 (ddd, 1H, J = 8.1, 7.1, 1.1 Hz, H-5‴), 6.87 (d, 2H, J = 8.7 Hz, H-2′,6′), 6.55 (br s, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 125.77 MHz): δ 193.7 (C-1″), 163.7 (C-2‴), 145.4 (C-β), 145.2, 144.1, 143.30, 143.26, 143.16, 142.7, 142.6, 142.1, 141.7, 141.2, 140.9, 140.8, 139.9, 135.9 (C-4‴), 133.7, 133.6, 133.57, 133.5, 132.9, 132.6, 132.1, 132.0, 131.72, 131.70, 131.0, 130.6, 129.5 (C-6‴), 128.9, 128.5, 127.86, 127.83, 126.79, 127.1, 126.93, 126.91, 127.0, 120.5, 120.2 (C-1‴), 118.9, 118.7 (C-5‴), 118.6 (C-3‴), 116.9 (C-α), 116.7, 115.8, 115.7, 114.5 (C-3); UV–Vis (DMF), λ_{max} (log ε): 277 (4.3), 414 (5.1), 538 (4.2), 585 (4.4) nm; HRMS ESI: m/z calcd for $C_{59}H_{39}N_5NiO_2$ [M$^{+\cdot}$]: 907.2457; found: 907.2452.
17. *Conjugate* ***11***: ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 9.07 and 9.02 (AB, 4H, J = 4.7 Hz, H-$\beta_{pyrrolic}$), 8.96 (s, 4H, H-$\beta_{pyrrolic}$), 8.26–8.19 (m, 10H, 5,10,15,20-H-*o*-Ph and 2 × H-5″), 8.01 (d, 4H, J = 8.9 Hz, 2 × H-3′,5′), 7.80–7.75 (m, 9H, 10,15,20-H-*m,p*-Ph), 7.75–7.70 (m, 2H, 2 × H-7″), 7.62–7.58 (m, 4H, 5-H-*m*-Ph and 2 × H-8″), 7.57 (d, 4H, J = 8.9 Hz, 2 × H-2′,6′), 7.46-7.41 (m, 2H, 2 × H-6″), 6.80 (s, 2H, 2 × H-3″); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 177.2 (C-4″), 167.3 (C-2″), 150.3, 145.6 (C-9″), 134.5, 133.7 (C-7″), 132.2, 132.12, 132.1, 131.6, 127.9, 127.5 (C-5″), 126.6 (C-6″), 125.2; 124.0 and 123.97 (C-8″ and C-10″), 123.8, 121.3, 121.27, 118.0, 106.6 (C-3″), 103.6; UV–Vis (DMF), λ_{max} (log ε): 306 (4.5), 426 (5.7), 559 (4.4), 600 (4.1) nm; HRMS ESI: m/z calcd for $C_{74}H_{45}N_5O_4Zn$ [M$^{+\cdot}$]: 1131.2750; found: 1131.2758.
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19. *Conjugate* ***10***: ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 9.05 and 8.96 (AB, 4H, J = 4.7 Hz, H-$\beta_{pyrrolic}$), 8.95 (br s, 4H, H-$\beta_{pyrrolic}$), 8.25–8.21 (m, 6H, 10,15,20-H-*o*-Ph), 8.20 (d, 2H, J = 8.4 Hz, 5-H-*o*-Ph), 8.15 (dd, 1H, J = 7.9, 1.5 Hz, H-5″), 7.96 (d, 2H, J = 8.8 Hz, H-3′,5′), 7.79–7.75 (m, 9H, 10,15,20-H-*m,p*-Ph), 7.72–7.66 (m, 1H, H-7″), 7.61–7.56 (m, 1H, H-8″), 7.58 (d, 2H, J = 8.4 Hz, 5-H-*m*-Ph), 7.47 (d, 2H, J = 8.8 Hz, H-2′,6′), 7.43–7.38 (m, 1H, H-6″), 6.70 (s, 1H, H-3″), 6.47 (br s, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 75.47 MHz): δ 179.4 (C-4″), 164.8 (C-2″), 156.3, 150.2, 150.13, 150.1, 147.9, 143.5, 143.4, 140.5 (C-9″), 137.7, 135.7, 134.6, 134.0 (C-7″), 131.7, 128.3, 127.3, 126.4, 125.43 (C-6″), 125.35 (C-5″), 123.7 (C-10″), 121.7, 120.7, 120.3, 120.1 (C-8″), 118.1, 117.6, 115.8, 104.6 (C-3″); UV–Vis (DMF), λ_{max} (log ε): 302 (4.5), 428 (5.6), 560 (4.3), 600 (4.2) nm; HRMS (ESI): m/z calcd for $C_{59}H_{37}N_5O_2Zn$ [M$^{+\cdot}$]: 911.2239; found: 911.2233.
20. *General procedure:* To a $CHCl_3$ solution of each metallo-conjugate was added 10% H_2SO_4 [for Ni(II) complex] or 5% TFA [for Zn(II)] and stirred at room temperature for about 20–30 min. The reaction mixture was poured into cold water, neutralized with a saturated aqueous solution of $NaHCO_3$, washed several times with water, and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. Further purification using preparative TLC, using a mixture of light petroleum and chloroform (1:2) as a solvent, afforded the desired free base conjugates.
21. *Conjugate* ***12***: ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 8.86 (AB, 1H, J = 4.9 Hz, H-$\beta_{pyrrolic}$), 8.82 (AB, 1H, J = 4.9 Hz, H-$\beta_{pyrrolic}$), 8.79 (AB, 1H, J = 4.9 Hz, H-$\beta_{pyrrolic}$), 8.76 (s, 2H, H-$\beta_{pyrrolic}$), 8.62 (AB, 1H, J = 4.9 Hz, H-$\beta_{pyrrolic}$), 8.43 (s, 1H, H-3), 8.25–8.17 (m, 9H, 5,10,15,20-H-*o*-Ph and H-5″), 8.00–7.93 (m, 1H, 20-H-*p*-Ph), 7.92–7.87 (m, 2H, 20-H-*m*-Ph), 7.86 (d, 2H, J = 8.8 Hz, H-3′,5′), 7.80–7.73 (m, 9H, 5,10,15-H-*m,p*-Ph), 7.72–7.67 (m, 1H, H-7″), 7.57 (dd, 1H, J = 8.4, 0.94 Hz, H-8″), 7.42 (ddd, 1H, J = 8.0, 7.0, 0.9 Hz, H-6″), 7.04 (d, 2H, J = 8.8 Hz, H-2′,6′), 6.82 (br s, 1H, N*H*), 6.76 (s, 1H, H-3″), −2.63 (br s, 2H, H-21 and H-23); ^{13}C NMR ($CDCl_3$, 75.47 MHz): δ 178.4 (C-4″), 163.4 (C-2″), 156.2 (C-9″), 145.5, 142.6, 142.1, 141.8, 140.8, 134.5, 134.4, 134.2, 133.5 (C-7″), 133.2, 129.9, 129.2, 128.6, 127.9, 127.8, 127.7, 126.9, 126.8, 126.7, 125.7, 125.0 (C-5″), 124.0 (C-6″), 123.0 (C-10″), 121.4, 120.2, 118.2, 117.9 (C-8″), 116.4, 115.7, 105.6 (C-3); UV–Vis (DMF), λ_{max} (log ε): 306 (4.3), 414 (5.3), 459 (4.8), 523 (4.4), 599 (4.0), 656 (3.5) nm; HRMS (ESI): m/z calcd for $C_{59}H_{40}N_5O_2$ [M+H]$^+$: 850.3173; found: 850.3177.
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