Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 4266–4270

Novel ebselen–porphyrin conjugates: Synthesis and nucleic acid interaction study

Zhi Xue,[a] An-Xin Hou,[a,*] Daniel Wei-Jing Kwong[b] and Wai-Kwok Wong[a,b,*]

[a]*Department of Chemistry, Wuhan University, Wuhan 430072, PR China*
[b]*Department of Chemistry, Hong Kong Baptist University, Hong Kong, PR China*

Received 4 February 2007; revised 10 April 2007; accepted 10 May 2007
Available online 26 May 2007

Abstract—Novel porphyrins bearing ebselen (2-phenyl-1,2-benzisoselenazol-3[2H]-one) moiety were synthesized and characterized. Their interactions with herring sperm DNA were studied by means of UV–visible, fluorescence, circular dichroism spectroscopy, and gel electrophoresis.

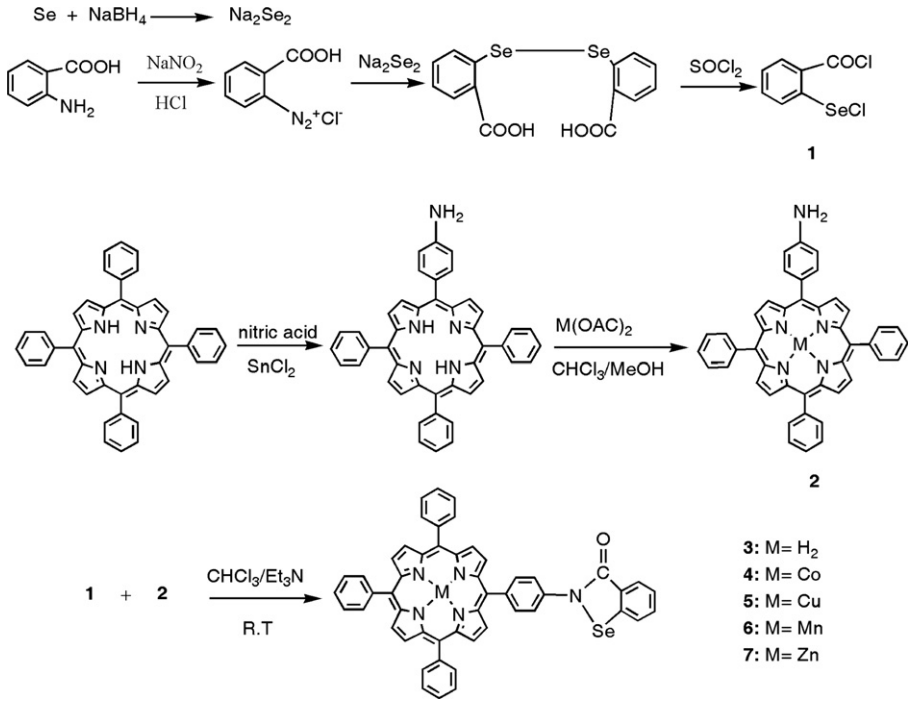
Investigations of porphyrin–nucleic acid interactions have been the subject of extensive research during the past decade and a great many of important results were achieved.[1] The study on porphyrin–nucleic acid interactions was largely motivated by such potential application as photodynamic inactivation of microorganisms and viruses, the probing of the secondary and tertiary structures of nucleic acids, and DNA-targeted antitumor activity.[2] For the porphyrin–DNA interaction, three binding modes (intercalative binding, external groove binding, and outside binding with self-stacking along the DNA helix) were widely accepted.[3] Previous studies have attributed the anticancer activities of cationic porphyrins to their intercalative binding with DNA.[4] But DNA-binding porphyrins included cationic, neutral as well as anionic porphyrins.[5] Thus, it is imperative to study the interactions of DNA with a wide variety of porphyrins. Results from these studies will be valuable toward the development of porphyrin-based compounds as novel DNA-targeted anti-tumor or antibiotic drugs.

Herein we report the synthesis of a series of novel ebselen–porphyrin conjugates and the results of their interactions with DNA, including their DNA photocleavage activities. The anti-oxidant, anti-inflammatory and anti-tumor activity of ebselen (2-phenyl-1,2-benzisoselenazol-3-[2H]-one) has been studied extensively.[6] The combination of porphyrin with ebselen might provide stronger anti-tumor activity due to the fact that porphyrins have specific affinity for tumor cells, which would enable the ebselen moiety to accumulate efficiently inside the tumor cells. Furthermore, it is reasonable to expect that the conjugate might display synergistic or additive anti-tumor effects due to the two moieties.

The synthetic route for 5-ebselenyl-10,15,20-triphenylporphyrin is shown in Scheme 1. Reduction of elemental selenium with potassium borohydride followed by treatment with diazotized anthranilic acid gave 2,2′-diselenobis(benzoic acid), which reacted with thionyl chloride to give 2-chloroselenobenzoyl chloride (**1**).[7] Meanwhile, the key of the synthesis is the preparation of 5-(4-aminophenyl)-10,15,20-triphenylporphyrin (**2**). In the classical Adler's condensation, neither 4-nitrobenzaldehyde nor 4-aminobenzaldehyde condensed with pyrrole to give **2**. However, **2** could be synthesized in good yield via nitration followed by reduction of tetraphenylporphyrin ($TPPH_2$).[8] Reaction between **1** and **2** gave the targeted conjugate **3**, which bore an ebselen moiety. The progress of the reaction was monitored by TLC. The targeted complex had a slightly higher R_f than **2**, in $CHCl_3$. The product **3** was air- and moisture-stable and could be purified by flash column chromatography. The metallo-ebselenylporphyrins (M = Co(II), Cu(II), Mn(II), and Zn(II)) were obtained by metalation of **2** first, then followed by reaction with **1**. The resulting metallo-ebselenylporphyrins (**4**–**7**) were readily soluble in DMF.

Keywords: Porphyrins; Ebselen; HS DNA; Binding mode.
* Corresponding authors. Tel.: +86 27 6875 2323; fax: +86 27 6875 4067 (A.-X.H.); tel.: +852 3411 7011; fax: +852 3411 7348 (W.-K.W.); e-mail addresses: houanxin@yahoo.com; wkwong@hkbu.edu.hk

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.05.074



Scheme 1. Synthetic routes for porphyrins with ebselen moiety.

ESI-MS analysis confirmed the metalation of the ebselen–porphyrin complexes. Due to the paramagnetic nature of porphyrins (**4**–**6**), their ^{1}H NMR spectral data were not available. The synthetic procedure and spectroscopic data of porphyrins **3** and **7** are given in the reference.[9]

The interaction of porphyrins **3**–**7** with herring sperm DNA (HS DNA) was evaluated using UV–visible absorption and fluorescence spectroscopy. To enhance solubility, the porphyrins were first dissolved in DMF and then diluted to 10 μM with deionized water. The diluted porphyrin solutions were titrated with increasing concentrations of HS DNA in Tris–HCl buffer and the volume percentage of DMF was kept to less than 5%. The concentration of the HS DNA solution, in terms of base pairs, was determined by its absorbance at 260 nm using the molar absorptivity, ε_{abs}, of $1.31 \times 10^4\ M^{-1}\ cm^{-1}$. The excitation ($\lambda_{ex}$) and emission ($\lambda_{em}$) wavelengths of the porphyrins were determined with the diluted porphyrin solutions (10 μM) in the absence of DNA. For **7**, the λ_{ex} and λ_{em} are 412 and 650 nm, respectively, in 0.05 M Tris–HCl buffer (pH 7.4) and 0.1 M NaCl. In the fluorescence titration experiments, after addition of DNA, the resultant solution was stirred vigorously and maintained at room temperature for 30 min before measurement was taken. The spectral changes upon DNA addition were different for different porphyrins, ranging from a modest 13% increase in intensity in **3** to ca. 185% enhancement in **6**. These results, together with the UV–visible spectral changes, are summarized in Table 1. In the UV–visible titration experiments, **3** and **5** gave no significant changes, **4** and **7** showed a relatively large red shift (9 nm and 15 nm, respectively) and hypochromicity (31.7% and 84.7%, respectively), and **6** gave a small red shift (2 nm) but a substantial hyperchromicity (66.6%). Among the three binding modes demonstrated for porphyrins, DNA intercalation is characterized by a large red shift (>10 nm) and a significant hypochromicity (up to 40%) in its Soret band; external groove binding by a minor (or no) spectral shift and an occasional hyperchromicity; and outside binding with self-stacking sharing similar characteristics with the intercalative binding mode.[2] According to this criterion, **7** appears to intercalate into DNA and **4** binds to DNA via outside binding with self-stacking. The remaining three ebselen–porphyrins seem to exhibit external groove binding mode. It is well known that the nature of the central metal ions of metalloporphyrins could influence their binding characteristics with DNA.[1b,10] This can partly explain the different binding modes shown by the five

Table 1. UV–visible and fluorescence spectral changes of the ebselen–porphyrins (**3**–**7**) upon titration with HS DNA

Porphyrins	UV–vis data of the Soret band		Fluorescence
	Red shift (nm)	Hypochromicity (%)	Intensity enhancement (%)
3	3	1.5	12.8
4	9	31.7	88.9
5	2	5.1	75.1
6	2	−66.6	184.2
7	15	84.7	38.5

The porphyrin–DNA mixture contained 10 μM of the ebselen–porphyrin and different molar ratios of DNA, $C_{DNA}/C_{porphyrin}$ = 0–10. A negative hypochromicity means a corresponding hyperchromicity. All experiments were performed at room temperature in buffer solution (0.05 M Tris–HCl, 0.1 M NaCl, pH = 7.4).

ebselen–porphyrins. The UV–visible titration spectra of **7** are shown in Figure 1.

Intercalative binding mode was characterized by large binding constant as well. The apparent binding constant (K_{app}) could be calculated according to Eq. (1):

$$\frac{[DNA]_{total}}{(|\varepsilon_{app}-\varepsilon_f|)} = \left\{\frac{1}{(|\varepsilon_b-\varepsilon_f|)}\right\}[DNA]_{total} + \frac{1}{\{K_{app}(|\varepsilon_b-\varepsilon_f|)\}} \quad (1)$$

where ε_{app}, ε_f and ε_b correspond to A_{obsd}/[porphyrin], the extinction coefficient for the free porphyrin, and the extinction coefficient for the porphyrin in the fully bound form, respectively. From a plot of $[DNA]_{total}/(|\varepsilon_{app}-\varepsilon_f|)$ versus $[DNA]_{total}$, shown in the inset of Figure 1, K_{app} is obtained by the ratio of the slope to the intercept.[11] K_{app} of **7** was determined to be $7.40\times10^5\ M^{-1}$, which compared favorably with the binding constant in the intercalative binding between TMPyP (TMPyP = tetrakis(4-methylpyridiniumyl)porphyrin) and poly[d(G-C)$_2$].[1b]

Fluorescence spectroscopy was also applied to study porphyrin–DNA interactions.[12] Fluorescence intensity of the five ebselen–porphyrins was enhanced to various extents upon addition of HS DNA. These data are given in Table 1. While four ebselen–porphyrins, **3**–**6**, showed only minor spectral shifts, $\lambda_{em,max}$ of **7** was substantially blue-shifted ($\Delta\lambda = 24$ nm) in the presence of DNA (Fig. 2). This unique feature might be indicative of the formation of a novel porphyrin–DNA complex. An enhanced fluorescence was once believed to be one of the criteria for intercalative binding.[1c,13] However, subsequent studies revealed that external groove binding showed similar changes as well.[14] Thus, intercalative binding mode could not be inferred solely on the basis of enhanced fluorescence upon DNA addition. Previous studies on the interactions of porphyrins and metalloporphyrins with DNA suggested that at low concentrations, the sign of the induced circular dichroism (CD) in

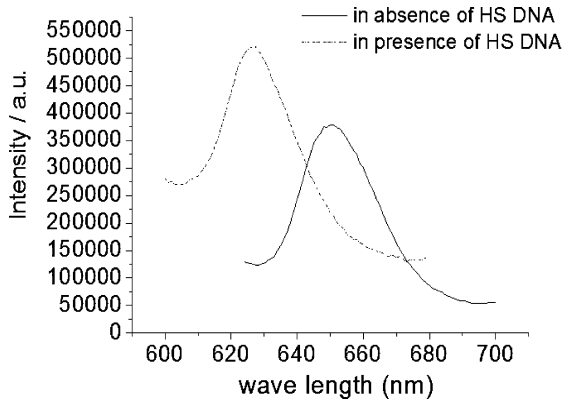


Figure 2. Fluorescence spectra ($\lambda_{ex} = 412$ nm) of **7** (10 μM) in the absence (—) and the presence (- - -) of HS DNA ($C_{DNA}/C_{porphyrin} = 10$) in 0.05 M Tris–HCl buffer (pH 7.4) and 0.1 M NaCl.

the Soret region of porphyrins can be used as a signature for its binding mode with DNA: a positive induced CD band is indicative of external groove binding, whereas a negative induced CD band is indicative of intercalative binding.[1b,15] To further investigate the binding modes, the CD spectra of these five porphyrins (**3**–**7**) upon addition of HS DNA were measured. Results showed that only **7** exhibited a negative CD signal in the Soret band (Fig. 3), while the other four porphyrins (**3**–**6**) exhibited a positive CD signal. These CD results corroborate with the results obtained from UV–vis and fluorescence titration experiments: in their binding interactions with HS DNA, **7** binds with an intercalative mode, while **3**–**6** bind externally, with **4** undergoing self-stacking along the DNA helix and **3**, **5**, and **6** presumably prefer groove binding with DNA.

DNA photocleavage activities of these ebselen–porphyrins were also measured using the plasmid DNA relaxation assay.[11] The gel image is given in Figure 4. At concentrations up to 20 μM (limited by their water solubility), only modest DNA photocleavage activities, ranging from 15 to 25%, were seen in the four ebse-

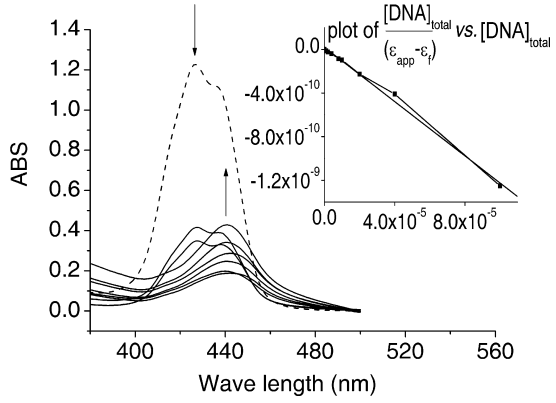


Figure 1. UV–visible spectra of porphyrin **7** (10 μM) in the absence (- - -) and presence (—) of increasing molar ratios of HS DNA ($C_{DNA}/C_{porphyrin}$ = 0, 0.1, 0.2, 0.4, 0.8, 1.0, 2.0, 4.0, 10.0). Hypochromism at 427 nm was observed at low $C_{DNA}/C_{porphyrin}$ ratios but red-shifted to near 440 nm at high $C_{DNA}/C_{porphyrin}$ ratio. The inset is a plot of $[DNA]_{total}/(|\varepsilon_{app}-\varepsilon_f|)$ versus $[DNA]_{total}$.

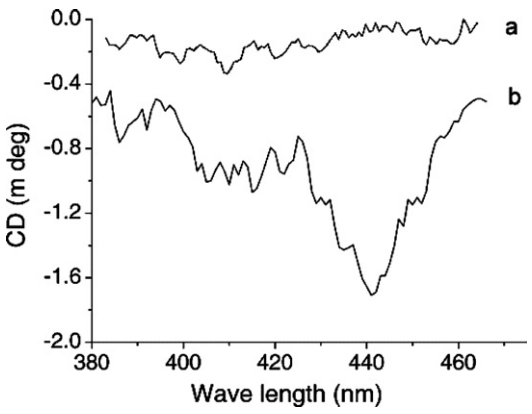


Figure 3. Induced circular dichroism spectrum of **7** with HS DNA (a) in the absence of DNA, (b) in the presence of DNA) [porphyrin] = 5 μM, $C_{DNA}/C_{porphyrin} = 20.0$. The spectrum was recorded in 0.05 M Tris–HCl buffer, pH = 7.4, and 0.1 M NaCl.

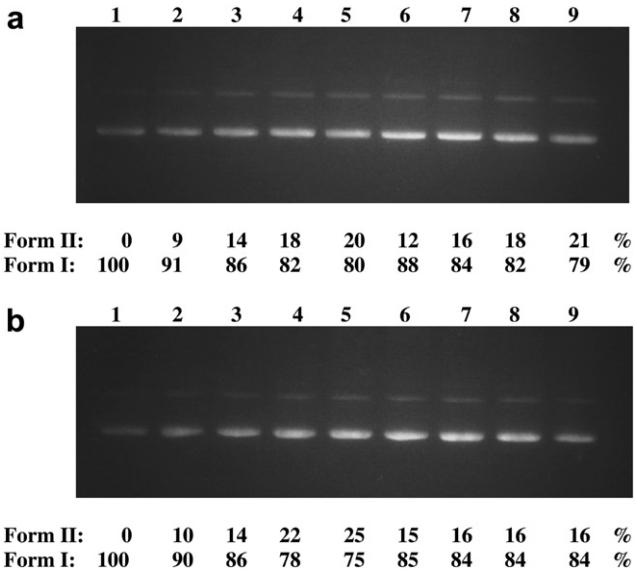


Figure 4. Agarose gel electrophoresis of DNA photocleavage assay of (a) porphyrin **7** and **3**; and (b) porphyrin **4** and **5**. In (a) lane 1: plasmid DNA (pBluescript); lane 2: **7** (1 μM) + DNA; lane 3: **7** (5 μM) + DNA; lane 4: **7** (10 μM) + DNA; lane 5: **7** (20 μM) + DNA; lane 6: **3** (1 μM) + DNA; lane 7: **3** (5 μM) + DNA; lane 8: **3** (10 μM) + DNA; lane 9: **3** (20 μM) + DNA. In (b) lane 1: plasmid DNA (pBluescript); lane 2: **5** (1 μM) + DNA; lane 3: **5** (5 μM) + DNA; lane 4: **5** (10 μM) + DNA; lane 5: **5** (20 μM) + DNA; lane 6: **4** (1 μM) + DNA; lane 7: **4** (5 μM) + DNA; lane 8: **4** (10 μM) + DNA; lane 9: **4** (20 μM) + DNA. Photo-irradiation was conducted using a transilluminator at 455 nm for 45 min under ambient temperature.

len–porphyrins studied, that is, porphyrin **4** (15%), **3** (20%), **7** (20%), and **5** (25%).

In summary, we have synthesized a series of metallo-ebselenylporphyrins. Using UV–vis, fluorescence, and circular dichroism techniques, their binding properties with HS DNA were investigated. While the metal-free ebselen–porphyrin and its Co(II), Cu(II), and Mn(II) complexes demonstrate outside binding with DNA, the Zn(II) complex showed an intercalative binding mode. This result stands in contrast to the more extensively studied meso-substituted cationic Zn(II)–TMPyP complex, which binds externally to the DNA, presumably because of the steric constraint from an axial aquo ligand.[1b] The reason for such distinct behavior was not apparent at present. However, it should be pointed out that two Zn(II) non-meso-substituted cationic porphyrins have recently been reported to bind to DNA via intercalation as well.[15] Despite the modest DNA photocleavage activities observed for **3, 4, 5, 7**, which only show that they are not efficient in causing DNA strand breaks, more definitive photodynamic assay using appropriate cancer cells will be conducted to properly evaluate the potential of these compounds as antitumor agents. With respect to some porphyrins bearing bioactive groups which exhibited efficient antibacterial activity,[16] these compounds will be also applied to other bioassay such as antibacterial or anti-inflammation activity.

Acknowledgments

W.-K.W. thanks the Hong Kong Baptist University (FRG/05-06/II-03) and Hong Kong Research Grants Council (HKBU 2021/03P) for financial support of this work. We thank the reviewers for their critical comments on the manuscript.

References and notes

1. (a) Fiel, R. J.; Howard, J. C.; Datta, G. N. *Nucleic Acids Res.* **1979**, *6*, 3093; (b) Pasternack, R. F.; Gibbs, E. J.; Villafranca, J. *Biochemistry* **1983**, *22*, 2406; (c) Sehlstedt, U.; Kim, S. K.; Carter, P.; Goodisman, J.; Vollano, J. F.; Norden, B.; Dabrowiak, J. C. *Biochemistry* **1994**, *33*, 417; (d) Sari, M. A.; Battioni, J. P.; Mansuy, D.; Le Pecq, J. B. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 643; (e) Ford, K. G.; Neidle, S. *Bioorg. Med. Chem.* **1995**, *3*, 671.
2. (a) Sol, V.; Branland, P.; Chaleix, V.; Granet, R.; Guilloton, M.; Lamarche, F.; Verneuil, B.; Krausz, P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4207; (b) Weisman-Shomer, P.; Cohen, E.; Hershco, I.; Khateb, S.; Wolfovitz-Barchad, O.; Hurley, L. H.; Fry, M. *Nucleic Acids Res.* **2003**, *31*, 3963; (c) Anton, B. G.; Neocles, B. L. *Biochemistry* **1999**, *38*, 15425.
3. (a) Fiel, R. J. *J. Biomol. Struct. Dynam.* **1989**, *6*, 1259; (b) Lugo-Ponce, P.; McMillin, D. R. *Coord. Chem. Rev.* **2000**, *208*, 169.
4. (a) Villanueva, A.; Juarranz, A.; Diaz, V.; Gomez, J.; Canete, M. *Anticancer Drug Des.* **1992**, *7*, 297; (b) Pasternack, R. F.; Gibbs, E. J. *Met. Ions Biol. Syst.* **1996**, *33*, 367.
5. (a) Balaz, M.; Steinkruger, J. D.; Ellestad, G. A.; Berova, N. *Org. Lett.* **2005**, *7*, 5613; (b) Lauceri, R.; Purrello, R.; Shetty, S. J.; Vicente, M. G. H. *J. Am. Chem. Soc.* **2001**, *123*, 5835.
6. (a) Moussaoui, S.; Obinu, M. C.; Daniel, N.; Reibaud, M.; Blanchard, V.; Imperato, A. *Exp. Neurol.* **2000**, *166*, 235; (b) Parnham, M.; Sies, H. *Expert Opin. Investig. Drugs* **2000**, *9*, 607.
7. Kamigata, N.; Izuka, H.; Izuoka, A.; Kobaysahi, M. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2179.
8. Kruper, W. J.; Chamberlin, T. A.; Kochanny, M. K. *J. Org. Chem.* **1989**, *54*, 2753.
9. Selected procedure: Under an argon atmosphere, fuming HNO_3 (0.32 g, 5.0 mmol, fuming) was added dropwise over a period of 20 min at 0 °C to a solution of tetraphenylporphyrin ($TPPH_2$) (1.0 g, 1.62 mmol) in $CHCl_3$ (150 cm^3). The reaction was completed within half an hour. The reaction mixture was then washed thrice with distilled water (3×100 cm^3) to remove the excess HNO_3. The $CHCl_3$ layer was then dried over $MgSO_4$, filtered, and evaporated to dryness. Then the crude product was chromatographed (silica gel: 100 g, eluent: $CHCl_3$/petroleum ether 2:1) and crystallized from methanol. The second band was gathered to give 5-(4-nitrophenyl)-10,15,20-triphenylporphyrin (p-NO_2-$TPPH_2$) in 50% yield. p-NO_2-$TPPH_2$ (2.00 g, 3.03 mmol) was dissolved in 60 cm^3 of concentrated hydrochloric acid under nitrogen. Tin(II) chloride dihydrate (2.08 g, 9.24 mmol) was added to the solution. The reaction solution was heated to 65 °C for 1 h and then poured into a beaker filled with ice for cooling. Sodium hydroxide was added to the mixture with vigorous stirring until the color of the solution turned red brownish. The solution was then extracted several times with chloroform. The combined chloroform solution was evaporated to dryness. The residue was purified by column

chromatography (silica gel: 200 g, eluent: $CHCl_3$/petroleum ether 4:1). Crystallization in methanol gave the free porphyrin NH_2-$TPPH_2$ in 60% yield. The corresponding metalloporphyrin was synthesized by the addition of excess $M(OAc)_2$ in methanol to the free porphyrin in chloroform at reflux, and purified by column chromatography. A chloroform solution containing compound **1** (30 mg, 0.12 mmol) and freshly distilled triethylamine (28 μL, 0.2 mmol)) was added dropwise to a chloroform solution of NH_2-$TPPH_2$ (50 mg, 0.08 mmol) at room temperature under an argon atmosphere. The progress of the reaction was monitored by TLC. When the starting material had completely reacted, the reaction mixture was washed by saturated $NaHCO_3$ solution and deionized water. The chloroform layer was separated and evaporated to dryness under vacuum. The crude product was purified by flash column chromatography to give **3** and metallo products (**4**–**7**). Selected data: compound **3**: **UV–vis** (CH_2Cl_2): λ_{max} (nm, $\log\varepsilon$) 418 (4.42), 368 (4.08); Fluorescence: λ_{ex} = 417 nm, λ_{em} = 646 nm; IR (KBr, cm^{-1}) 1606 (C═O); HRMS (ESI, *m*/*z*) (calculated 811.1850, found 811.1901); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.85–8.93 (8H, pyrrolic), δ 7.56-8.30 (23H, ArH), δ −2.77 (inner NH). Compound **7**: UV–vis (CH_2Cl_2): λ_{max} (nm, $\log\varepsilon$) 548 (4.11), 421 (5.63); Fluorescence (H_2O): λ_{ex} = 412 nm, λ_{em} = 650 nm; IR (KBr, cm^{-1}) 1597 (C═O); HRMS (ESI, *m*/*z*) (calculated 873.0895, found 873.1013); ^1H NMR (DMSO-d_6, 300 MHz): δ 8.75–8.91 (8H, pyrrolic), δ 6.97–8.18 (23H, ArH).

10. Daryono, H. T.; Shunsuke, M.; Takehiro, A.; Naoki, Y.; Hidenari, I. *J. Inorg. Biochem.* **2001**, *85*, 219.
11. Mettath, S.; Munson, B. R.; Pandey, R. K. *Bioconjug. Chem.* **1999**, *10*, 94.
12. (a) Le Pecq, J. B.; Paoletti, J. B. C. *J. Mol. Biol.* **1967**, *27*, 87; (b) Pasternack, R. F.; Caccam, M.; Keogh, B.; Stephenson, T. A.; Williams, A. P.; Gibbs, E. J. *J. Am. Chem. Soc.* **1991**, *113*, 6835.
13. (a) Sari, M. A.; Battioni, J. P.; Duprt, D.; Mansuy, D.; Le Pecq, J. B. *Biochemistry* **1990**, *29*, 4205; (b) Zupan, K.; Herenyi, L.; Toth, K.; Majer, Z.; Csik, G. *Biochemistry* **2004**, *43*, 9151.
14. Hyun, K. M.; Choi, S. D.; Lee, S.; Kim, S. K. *Biochim. Biophys. Acta* **1997**, *1334*, 312.
15. Yellappa, S.; Seetharamappa, J.; Rogers, L. M.; Chitta, R.; Singhal, R. P.; D'Souza, F. *Bioconjug. Chem.* **2006**, *17*, 1418.
16. (a) Li, H. P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6298; (b) Zhang, J. Y.; Wu, X. J.; Cao, X. P.; Yang, F.; Wang, J. F.; Zhou, X.; Zhang, X. L. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1097.