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Bioorganic & Medicinal Chemistry Letters 18 (2008) 2152–2155

Bioorganic & Medicinal Chemistry Letters

Photochemical DNA cleavage by novel water-soluble sulfonated dihydroxy phosphorus(V) tetrabenzotriazacorrole

Lei Huang, Cheng Zhong, Fushi Zhang* and Chen-Ho Tung*

The Key Lab of Organic Photoelectrons & Molecular Engineering of Ministry of Education, Department of Chemistry, Tsinghua University, Haidian, Beijing 100084, People's Republic of China

Received 2 November 2007; revised 18 January 2008; accepted 21 January 2008
Available online 2 February 2008

Abstract—The photosensitizing properties of a novel water-soluble phthalocyanine-like photosensitizer, sulfonated dihydroxy phosphorus(V) tetrabenzotriazacorrole {$P(OH)_2TBCS_n$}, have been reported. It is relevant for the use of this dye as photodynamic sensitizer. The compound exhibited significant photocleavage of supercoiled (SC) pUC19 DNA. The photooxygenation and photocleavage to DNA showed high efficiency in generating singlet oxygen.

Phthalocyanines are the second generation photosensitizers which are synthesized to overcome the disadvantages of porphyrin derivatives and received considerable attention due to their possible uses in photodynamic treatment of several diseases.[1,2] Compared with porphyrins, phthalocyanines exhibit advantageous photophysical properties for photodynamic therapy (PDT) including a high molar absorption in the red portion of the visible spectrum, long lifetime of triplet state and high efficiency to generate singlet oxygen ($^1O_2$), which is the main cause for the cytotoxic effect in the cell deactivation.[3–5] Metalloid phthalocyanines are recently attracting great interests owing to their potential applications in photodynamic treatment.[1,6] Phosphorus plays a crucial role in human metabolism. And it is the smallest ion which could be found to insert into a tetrapyrrolic ring. When coordinated with phosphorus, phthalocyanine may lose a bridging nitrogen atom, which formed the complex of tetrabenzotriazacorrole (TBC).[7–10] The dihydroxy phosphorus(V) tetrabenzotriazacorrole [$P(OH)_2TBC$] has a non-planar structure. Thus, dihydroxy phosphorus(V) tetrabenzotriazacorrole derivative provides a novel species to these macrocyclic compounds, with novel chemical and spectroscopic properties.[3,11] Due to the desirable photophysical properties and promising results found in vitro or in vivo photosensitizing efficiencies of the phosphorus(V) tetrabenzotriazacorrole, there has been increasing interests in the synthesis of new phosphorus(V) tetrabenzotriazacorrole.

It is known that porphyrin as a photosensitizer can localize in tumor cells and be phototriggered to produce singlet oxygen to cleave DNA and damage tumor cells.[12] In contrast to porphyrin, there are only a few reports on studies of the DNA cleavage by phthalocyanines. In the present work, we report our first results of the DNA photocleavage by $P(OH)_2TBC$ derivative. Water-soluble $P(OH)_2TBC$ derivative was synthesized to facilitate better absorption by tumor tissues for the application in PDT. The photosensitizing properties of this phthalocyanine-like photosensitizer in DMSO were also studied.

The target compounds $P(OH)_2TBC$ and $P(OH)_2TBCS_n$ were synthesized according to the procedures described in Scheme 1. The commercially available metal-free phthalocyanine (H_2Pc) was reacted with the PBr_3 in pyridine to afford the $P(OH)_2TBC$.[9] $P(OH)_2TBCS_n$ was obtained by treatment of $P(OH)_2TBC$ in fuming sulfuric acid (30% SO_3). The results were consistent with literature data.[13]

The photophysical properties that are required for dihydroxy phosphorus(V) tetrabenzotriazacorroles as photosensitizer include high triplet yield (Φ_T), and high singlet oxygen quantum yield (Φ_Δ) as well as long triplet lifetime (τ_T). These parameters are of particular importance in PDT. To confirm the efficiency of the compounds,

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Keywords: Tetrabenzotriazacorrole; Phosphorus; DNA; Cleavage.

* Corresponding authors. Tel.: +86 10 62782596; fax: +86 10 62770304; e-mail addresses: zhangfs@mail.tsinghua.edu.cn; chtung@mail.ipc.ac.cn

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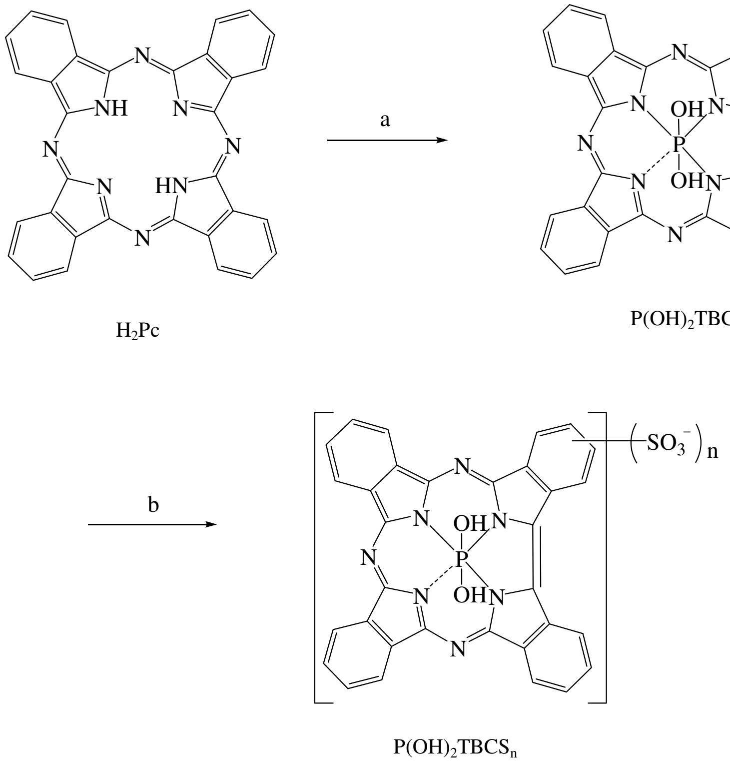
doi:10.1016/j.bmcl.2008.01.111



Scheme 1. Synthesis of dihydroxy phosphorus(V) tetrabenzotriazacorrole and sulfonated dihydroxy phosphorus(V) tetrabenzotriazacorrole. Reagents and conditions: (a) PBr_3, pyridine (100 °C, 6 h), yield: 67%; (b) fuming sulfuric acid (80 °C, 2 h), yield: 47%.

triplet absorption spectra, triplet lifetimes, and quantum yields of singlet oxygen formation were measured on a nanosecond flash photolysis apparatus. The triplet quantum yields (Φ_T) of $P(OH)_2TBC$ and $P(OH)_2TBCS_n$ were determined by comparative method[14] according to Eq. 1. Tetraphenylporphyrin (TPP) was used as a standard.

$$\Phi_T^{Sample}\varepsilon_T^{Sample} = \frac{\Delta A_T^{Sample}}{\Delta A_T^{Std}}\Phi_T^{Std}\varepsilon_T^{Std}, \tag{1}$$

where ΔA_T^{Sample} and ΔA_T^{Std} are the changes in the triplet state absorbance of the $P(OH)_2TBC$ derivative and the standard, respectively. ε_T^{Sample} and ε_T^{Std} are the triplet state molar extinction coefficients for the $P(OH)_2TBC$ derivative and the standard, respectively. Φ_T^{Std} is the triplet state quantum yield for the standard, which is TPP in benzene ($\Phi_T = 0.82$).[15] ε_T^{Sample} was determined by the singlet depletion method[16] according to the equation:

$$\varepsilon_T = OD_T^{\infty}/N_S d, \tag{2}$$

where N_S is the concentration of the sample and d is the thickness of the sample. OD_T^{∞} is the saturated absorbance of T–T transition at the maximum wavelength of sample.

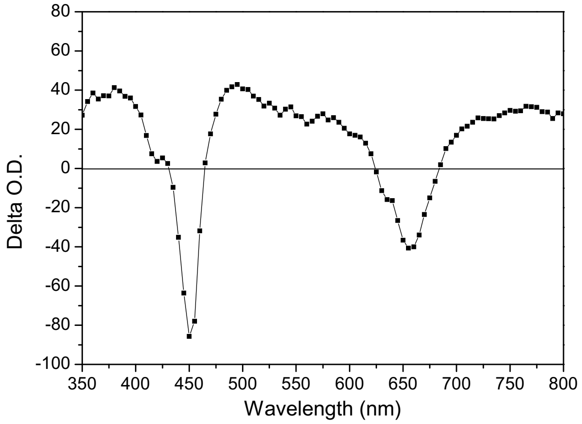


Figure 1. Transient absorption spectra of nitrogen-saturated solution of $P(OH)_2TBCS_n$ in DMSO ($\lambda_{ex} = 355$ nm) at room temperature.

Nitrogen-saturated $P(OH)_2TBCS_n$ in DMSO, upon excitation with 7 ns pulses of 355 nm laser, showed a broad transient absorption in the visible region from 350 to 800 nm with a maximum value at 495 nm (Fig. 1). The photobleaching of the B-band is from 430 to 465 nm and the photobleaching of Q-band is from 625 to 685 nm. The triplet state properties of $P(OH)_2TBC$ are summarized in Table 1. The triplet quantum yield of $P(OH)_2TBCS_n$ ($\Phi_T = 0.64$) in DMSO is higher than that of $P(OH)_2TBC$ ($\Phi_T = .44$). However, it is striking from Table 1 that sulfonation brought about a longer triplet lifetime. This implies, as evidence

Table 1. Photophysical parameters of $P(OH)_2TBC$ and $P(OH)_2TBCS_n$[a]

Compound	Φ_T	$\tau_T/\mu s$	Φ_Δ
$P(OH)_2TBC$	0.44	91	0.21
$P(OH)_2TBCS_n$	0.64	234	0.88

[a] Relative error is 10%.

by the smaller deviation from Beer's law (Supplementary information), that the anionic complexes are less efficient to form aggregates because of the enhanced solubility. It has already been known that the aggregation behavior may quench the excited states.[17]

An essential parameter relating to photosensitizing efficiency is the quantum yield of the formation of singlet oxygen, which was determined by the time-resolved IR illuminescence technique using ZnPc in DMSO ($\Phi_\Delta = 0.67$) as standards.[18] Air-equilibrated solutions of $P(OH)_2TBC$ and $P(OH)_2TBCS_n$ in DMSO were examined by detecting the luminescence in the near-infrared at 1270 nm upon excitation by 7 ns pulses of 355 nm laser, where singlet oxygen phosphoresces. Compared to $P(OH)_2TBC$ in DMSO ($\Phi_\Delta = 0.21$), the singlet oxygen quantum yield of $P(OH)_2TBCS_n$ in DMSO ($\Phi_\Delta = 0.88$) is higher. It shows that the sulfonation of $P(OH)_2TBC$ remarkably improved the triplet quantum yield and the singlet oxygen quantum yield. The increasing sequence of Φ_Δ matches well with that of the value of the triplet state quantum yield. The lifetime of singlet oxygen luminescence for all these dihydroxy phosphorus(V) tetrabenzotriazacorroles in DMSO solution was found to be very close (30 μs).

One of the major targets for the photodynamic action of phthalocyanate is DNA. The DNA photocleavage activity by dihydroxy phosphorus(V) tetrabenzotriazacorroles was examined using supercoiled double-stranded pUC19 plasmid DNA. A mixture of $P(OH)_2TBC$ (or $P(OH)_2TBCS_n$) in DMSO and the plasmid DNA in Tris–HCl buffer (pH 7.4) was illuminated in air at room temperature under red light (300W, tungsten lamp, >610 nm, 10 cm away from the tested tubes). After illumination, conversion of supercoiled DNA (form I) to nicked circular DNA (form II) was visualized by agarose gel electrophoresis and subsequent ethidium bromide staining. Results of DNA cleavage by $P(OH)_2TBC$ and $P(OH)_2TBCS_n$ were illustrated in Figures 2 and 3, respectively.

No DNA scission was observed when the photosensitizer was kept in the dark even though $P(OH)_2TBC$ or $P(OH)_2TBCS_n$ was present (lanes 2, 4, 6, 8 in Fig. 2 and lanes 1, 3, 5, 7 in Fig. 3). Lanes 5, 7, and 9 in Figure 2 demonstrate the concentration-dependent photocleavage by $P(OH)_2TBC$. The intensity of form I is decreased by higher concentration of $P(OH)_2TBC$. Similarly, lanes 2, 4, and 6 in Figure 3 show the effect of concentration of $P(OH)_2TBCS_n$ on the DNA cleavage. Thus a concentration-dependent DNA scission is observed for both $P(OH)_2TBC$ and $P(OH)_2TBCS_n$. In Figure 2, it is observed that the irradiated $P(OH)_2TBC$ (100 μM) completely degraded form I DNA. And in Figure 3 form I DNA is completely degraded by $P(OH)_2TBCS_n$ (50 μM) after being irradiated. Comparison of lane 7 in Figure 2 ($P(OH)_2TBC$) and lane 6 in Figure 3 ($P(OH)_2TBCS_n$) shows that $P(OH)_2TBCS_n$ exhibits greater photodamage. These observations correlate well with the higher $^1O_2$ generating efficiency of $P(OH)_2TBCS_n$. The role of $^1O_2$ has been well demonstrated in the in vitro photoactivated DNA scission. Therefore, this water-soluble phthalocyanine-like photosensitizer had high activity for DNA scission with visible light in Tris–HCl buffer.

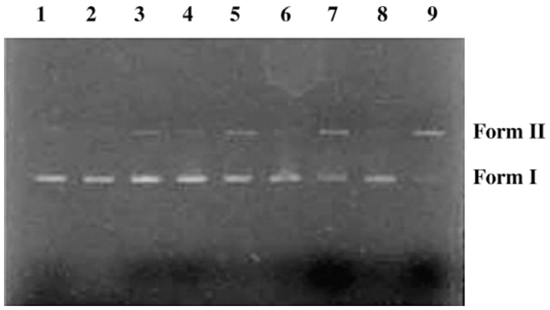


Figure 2. Agarose gel electrophoresis pattern for the cleavage of supercoiled pUC19 DNA by $P(OH)_2TBC$. Reaction mixtures (10 μL) contained 0.2 μg of plasmid DNA. Lane 1: DNA + 2.5% DMSO; lane 2: DNA + $P(OH)_2TBC$ (5 μM) + 2.5% DMSO; lane 3: DNA + $P(OH)_2TBC$ (5 μM) + 2.5% DMSO + hγ (30 min); lane 4: DNA + $P(OH)_2TBC$ (10 μM) + 2.5% DMSO; lane 5: DNA + $P(OH)_2TBC$ (10 μM) + 2.5% DMSO + hγ (30 min); lane 6: DNA + $P(OH)_2TBC$ (50 μM) + 2.5% DMSO; lane 7: DNA + $P(OH)_2TBC$ (50 μM) + 2.5% DMSO + hγ (30 min); lane 8: DNA + $P(OH)_2TBC$ (100 μM) + 2.5% DMSO; lane 9: DNA + $P(OH)_2TBC$ (100 μM) + 2.5% DMSO + hγ (30 min).

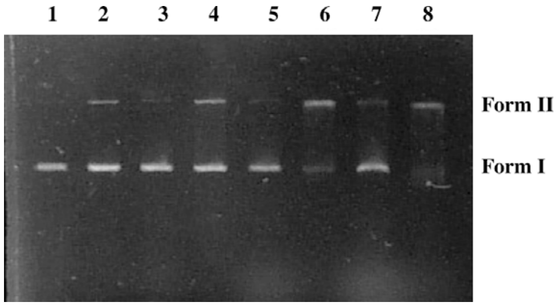


Figure 3. Agarose gel electrophoresis pattern for the cleavage of supercoiled pUC19 DNA by $P(OH)_2TBCS_n$. Reaction mixtures (10 μL) contained 0.2 μg of plasmid DNA. Lane 1: DNA + $P(OH)_2TBCS_n$ (5 μM) + 2.5% DMSO; lane 2: DNA + $P(OH)_2TBCS_n$ (5 μM) + 2.5% DMSO + hγ (30 min); lane 3: DNA + $P(OH)_2TBCS_n$ (10 μM) + 2.5% DMSO; lane 4: DNA + $P(OH)_2TBCS_n$ (10 μM) + 2.5% DMSO + hγ (30 min); lane 5: DNA + $P(OH)_2TBCS_n$ (50 μM) + 2.5% DMSO; lane 6: DNA + $P(OH)_2TBCS_n$ (50 μM) + 2.5% DMSO + hγ (30 min); lane 7: DNA + $P(OH)_2TBCS_n$ (100 μM) + 2.5% DMSO; lane 8: DNA + $P(OH)_2TBCS_n$ (100 μM) + 2.5% DMSO + hγ (30 min).

In conclusion, new water-soluble phthalocyanine-like photosensitizer, $P(OH)_2TBCS_n$, was synthesized and characterized. The photophysical properties of this novel macrocyclic compound have been studied. The triplet quantum yield of $P(OH)_2TBCS_n$ is moderately high in DMSO. Sulfonation increases both Φ_T and τ_T. It is likely that Type II photosensitization predominates in DMSO solution.

The compound also exhibits efficient singlet oxygen generation. The Φ_Δ of $P(OH)_2TBCS_n$ is higher than that of $P(OH)_2TBC$ in DMSO. It shows that the sulfonation of $P(OH)_2TBC$ remarkably improved the singlet oxygen quantum yield. The $P(OH)_2TBCS_n$ is capable of DNA cleavage with higher efficiency than $P(OH)_2TBC$ upon irradiation with visible light. Furthermore, the phosphorus compounds have exhibited excellent specific DNA oxidation cleavage in human cells.[19,20] Taking this into account, further research on the $P(OH)_2TBCS_n$ photocytotoxicities for cancer cell is worthwhile.

Acknowledgments

This work was supported by the National Natural Science General Foundation of China (Grants 20773077, 20572059, 20502013), and National Key Fundamental Research Program (2007CB808000).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.01.111.

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