Synthesis and Photocytotoxicity of Nitroxyl Radical-substituted Porphyrin

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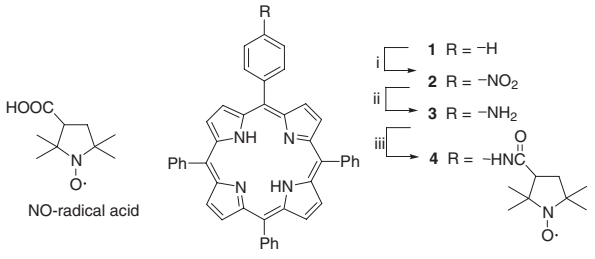
A novel tetraphenylporphyrin derivative bearing nitroxyl radical moiety was efficiently synthesized by improved preparation of monoamino-substituted tetraphenylporphyrin as the precursor. Preliminary photocytotoxicity investigation of the spin labeled porphyrin against SPC-A1 adenocarcinoma cell lines was tested.

In the past decades, a wide variety of structurally modified porphyrins have been synthesized for their potential application as photosensitizer in photodynamic therapy (PDT) of cancer.[1] In attempt to improve tumor cell targeting, efforts have been directed at covalently attaching porphyrins to a number of biomolecules including sugar[2,3] and peptide.[4] It has been reported that a number of nitroxyl spin labeled derivatives of antitumor compounds had superior pharmacological properties and marked decrease in toxicity compared to their parent molecules.[5,6] Although the exact mechanism is unknown, nitroxyl radical derivatives was thought to be beneficial for the drug to permeate through the cell membrane to reach the DNA.[7] Thus, it is of interest to attain nitroxyl radical-containing porphyrin for biological investigation.

meso-Tetraphenylporphyrin[8] (TPP, **1**) is one of the most readily available synthetic porphyrin. Its monofunctinalized derivatives such as **3** could serve as useful synthetic precursors for further modification. However, significant difficulty in preparation of **3** was caused by the low yield and tedious separation with classical procedure of condensation between pyrrole and two different aldehydes.[9] Previously Kruper et al.[10] reported mononitration at phenyl ring of TPP with excess of fuming nitric acid. Now we report our exploitation of this reaction to synthesize mono-nitroxyl radical-substituted tetraphenylporphyrin **4**. Its in vitro photocytotoxicity against SPC-A1 adenocarcinoma cell line was tested.

The synthesis procedure was shown in Scheme 1. The starting porphyrin TPP was subjected to regiospecific nitration to give 5-(4-nitrophenyl)-10,15,20-triphenylporphyrin **2**. It was found that the reaction mainly depends on the concentration and stoichiometry of the nitric acid used, as shown in Table 1.



Scheme 1. Synthesis of nitroxyl radical-substituted porphyrin. i) concentrated nitric acid ($d = 1.4\,g\,mL^{-1}$), 77%; ii) $SnCl_2$, HCl, 83%; iii) NO- radical acid, DCC, CH_2Cl_2, 90%.

Instead of the fuming nitric acid ($d = 1.5\,g\,mL^{-1}$) which was used in the Kruper's method, less-concentrated nitric acid ($d = 1.4\,g\,mL^{-1}$) was found to be much preferable for this reaction in the yield high as 77%.

Table 1. Regiospecific nitration of TPP with nitric acid[a]

Nitration Reagents	Stoichiometry of Nitric Acid	Yield[b] of **2**
Concentrated nitric acid ($d = 1.4\,g\,mL^{-1}$)	17	49
	19	64
	23	77
	25	71
	50	62
Fuming nitric acid ($d = 1.5\,g\,mL^{-1}$)	17	37
	19	45
	23	29
	30	3

[a] In CH_2Cl_2 at 0–5 °C under N_2 for 3 h. [b] Percent isolated yield was determined by silica gel chromatography.

Usual reduction of the nitro group with $SnCl_2$/HCl was applied to **2** to give 5-(4-aminophehyl)-10,15,20-triphenylporphyrin **3**. Followed by the condensation reaction with 1-oxyl-2,2,5,5-tetramethylpyrrolidine-3-carboxylic acid[11] in the presence of dicyclohexylcarbodiimide (DCC) in CH_2Cl_2, porphyrin **3** was converted to the nitroxyl radical-substituted porphyrin **4**.[12]

The photocytotoxicity of porphyrin **4** was tested against SPC-A1 adenocarcinona cell line. A normal cell line, L929 mouse fibroblast cell line, was also applied to the test for comparison. Cells were suspended in a RPMI medium containing 10^{-4} M porphyrin. The suspension was irradiated with fluorescent light ($\lambda = 600$ nm, fluence rate $= 60\,W\,m^{-2}$) for a certain period of time. After further 24 h incubation in dark at 37 °C, the dead cells were identified as propidium iodide (PI) permeable ones, and the counts were measured by flow cytometry.

Figure 1 displays dead cell counts in function of irradiation time with porphyrins **3** and **4** against SPC-A1 cells and L929 mouse fibroblast cells, respectively. The dead cell percentage increased with augmentation of irradiation time for both of the porphyrins. The dead SPC-A1 cell counts were always higher than that of L929 cells in the same irradiation time. In comparison with its precursor **3**, the nitroxyl radical-substituted porphyrin **4** exhibited enhanced photocytotoxicity and selectivity against tumor cell, and the maximum of dead SPC-A1 cell percentage (87%, 140 min) was over twice of that of L929 cell. It might be attributed to the superior membrane permeability arising from the nitroxyl radical-substitution.[7]

In conclusion, a novel tetraphenylporphyrin derivative bearing nitroxyl radical moiety was synthesized through three steps with high yield. Introduction of nitroxyl radical group to porphyrin was found to be beneficial for the photocytotoxicity against

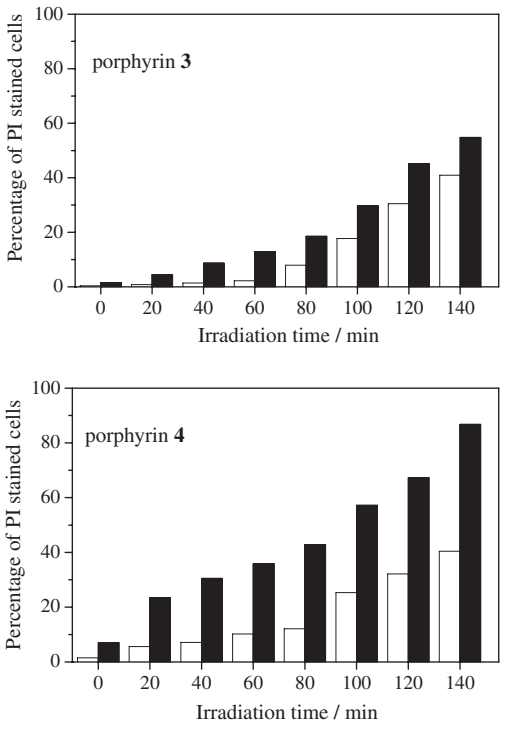


Figure 1. Irradiation time *vs.* percentage of PI stained L929 cells (void bars) and SPC-A1 cells (solid bars).

cancer cell, and therefore worthy to be further studied.

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12 Spectroscopic data for porphyrin **4**: ESR (1×10^{-4} mol L^{-1} in $CHCl_3$): **3** lines, $g_0 = 2.0019$, $A_N = 15.8$ Gs, $H_0 = 1.8$ Gs; UV: λ_{max} ($CHCl_3$) 421, 517, 552, 591, 650 nm; IR (KBr): 3434, 1670(C=O), 1596, 1508, 1440, 1400, 1350, 1290, 1243 cm^{-1}; Anal. Calcd for $C_{53}H_{45}N_6O_2$: C, 79.86; H, 5.68; N, 10.53; Found: C, 79.80; H, 5.65; N, 10.54%.

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